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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

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BIOLOGICAL BULLETIN

AUTOTOMY OF THE TAIL IN RODENTS.

F. B. SUMNER AND H. H. COLLINS.

It is perhaps not generally known that certain species of small rodents detach portions of the tail in a seemingly voluntary manner when they are seized by this member. Among the mouse-like rodents of California such an autotomy of the tail appears to be most typically exhibited by the pocket-mice (*Perognathus*).

It is the general practice of the writers, as probably of most breeders of mice, to pick up their animals by the tail. This appendage constitutes a convenient handle by which a mouse may be lifted, commonly relieving one of the risk of being bitten. But such a procedure is hazardous in the case of *Perognathus*. By a sudden gyratory movement of the body, the tail is likely to be severed at some point in its length, allowing the animal to make its escape and giving the pursuer something of a handicap in the race.

The whole performance is so suggestive of what occurs in many lizards that the question naturally arose in our minds: Can it be possible that autotomy, in the one case, as in the other, is followed by regeneration? This of course was not to be expected in a mammal, but the experiment seemed at least worth trying. In any case, the act and its consequences were thought to be worthy of a brief consideration.

The species upon which most of these observations were conducted was a rather large pocket-mouse, *Perognathus fallax fallax* Merriam, which is very common in the neighborhood of La Jolla. Despite the frequency of autotomy in this species, we soon found that all individuals cannot be depended upon to "perform" when desired. Those, in particular, which have been kept captive for some time seem to lose the tendency. Moreover, the act, when it does occur, is so sudden that one is commonly left wondering how it happened. But it was watched rather carefully in a number of instances, in order that the movements might be noted. In some cases, the body seemed to undergo a rapid whirling motion, comparable with that of a rope tied at one end to a post and held by the hand at the other. The snout and tail, in this comparison, correspond to the points of attachment, the hind quarters of the body to the whirling central portion of the rope. A number of individuals which did not actually detach the tail were found to undergo less violent gyrations of the same sort. In other instances, however, there seemed to be an actual torsion of the appendage, resulting from a rotation of the body. In yet another, where the tail was held down upon a cement floor, the detachment was effected by a sudden leap.

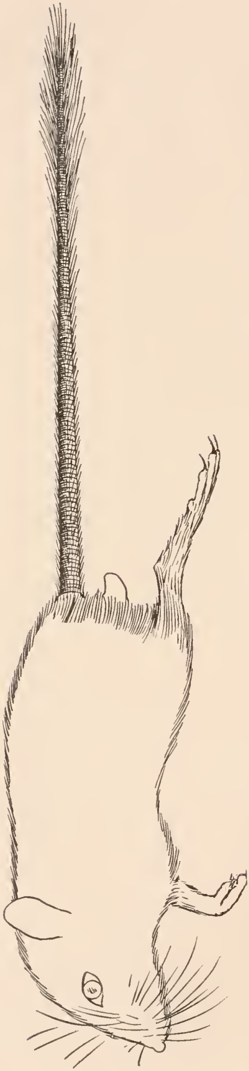

Fig. 1. *Perognathus fallax fallax*, showing normal condition of tail (somewhat reduced).

In eight cases, in which the detached portions of the appendage were carefully examined, it was found that the fracture invariably occurred across one of the caudal vertebræ, commonly near its center. The tail is likely to be broken at almost any point in its length. The detached portion carries away with it a group of tendons of such length as to indicate that these have their points of origin within the body itself. The possibility of a regeneration of the missing part of the tail was tested in fourteen specimens. Some of these underwent a voluntary autotomy, in the manner above described. In others, the breaking of the appendage was facilitated by the observer. These mice were all nearly mature, though probably most of them had not reached the limit of growth.

In the first seven specimens, the stump of the tail was measured shortly after the operation, but the length of the body was unfortunately not taken. Four of these specimens were living at the end of twelve months. In three cases, the tail has undergone a slight elongation (1–5 mm.), but this was doubtless merely an ordinary process of growth, accompanying the general growth of the body. In the fourth case, the recorded growth was 18 mm, though we believe this to be based upon an error in the first measurement. Except for the hair conditions, to be described below, there was no evidence of restoration of any part of the tail.

The next seven specimens were dealt with much more carefully. The length of the detached portion of the appendage was determined; likewise that of the stump, and the length of the body from the snout to the base of the tail.[1] Six of this second lot of mice were living at the end of nine months. When measured then, it was found that the tail stumps in five specimens had undergone a trivial increase in length, averaging about two millimeters. This was evidently incidental to a slight general growth of the body, since the mean body length, during the interval, had increased about four millimeters. In the sixth specimen, the tail was actually shorter, its condition indicating that it had been further damaged after the original operation.

Examination of the tissues of the tail in all of the foregoing specimens gave no suggestion of regenerative processes. In each case, the vertebral column terminated in a partial vertebra, this being evidently the one which was fractured in the process of autotomy.

There was, however, one very interesting result. In most of the specimens a dense tuft of elongated hairs had formed at the termination of the stump, the solid core of the latter being in some instances enlarged at the end. This tuft was entirely a new growth, since the middle region of the tail is normally covered with very short hair. As the uninjured tail of this rodent terminates in a "pencil" or tuft of longer hairs (Fig. 1) the formation of such a tuft at the point of amputation gave the appearance of a restorative process. There are, however, considerable points of difference between the normal "pencil" and the secondary one. The former is produced by the gradual elongation of the hair which covers the tail, commencing with the posterior third or half of this member. The restored tuft (Fig. 2) commences much more abruptly, sometimes being confined to the end of the stump. Its hairs are much longer than those of the natural pencil, and their pigmentation is conspicuously decreased, being pale gray on the normally black dorsal side.

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[1] In making the latter measurements the animal was etherized.

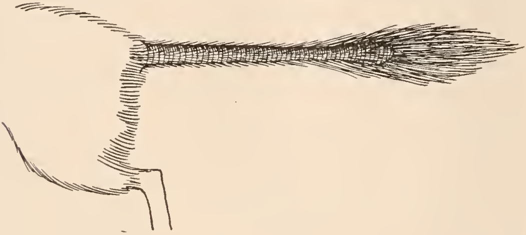

FIG. 2. Tail which has undergone autotomy about midway in its length, and upon which a new terminal tuft of hair has been produced.

In two instances a small lateral tuft was likewise formed, not far from the end of the stump. In one of these cases, there was found to be an internal injury, corresponding in position to this lateral tuft.

The capture of two wild specimens with truncated tails indicates that this accident may not be uncommon in nature. There was, of course, no clue to the source of the injury. Both specimens had developed terminal tufts, one of these being unusually perfect.

The time required for the formation of a new "pencil" has not been definitely noted. We have record of its having arisen within two months, but we also know that more than four months may be necessary.

The writers offer no speculations as to the physico-chemical factors which bring about this excessive growth of the hair on the severed stump of the tail of *Perognathus*. A superficial analogy suggests itself between this terminal tuft and the cluster of adventitious shoots which arise from the stump of a tree. Possibly the analogy is more than superficial.

How general this process of autotomy is among the pocket-mice we can not say. We have noted its occurrence in only one other species, *P. panamintinus bangsi*, a small desert form in which the process is closely similar to that in *P. fallax*.

To what extent this phenomenon may be spoken of as a "protective adaptation," and whether or not it arose through natural selection, we are unwilling to conjecture. It is more than possible that a pursuing carnivore would sometimes be cheated of its prey in this manner, since the greatly elongated tail might readily be seized by the claws or teeth. The association between the fragile structure of this appendage and the peculiar instinctive responses of the animal is probably not accidental.

A phenomenon similar to that discussed above, but differing in essential features, has been observed by one of us in a species of *Peromyscus* (*P. boylei rowleyi*). This mouse likewise has a relatively long tail, and undergoes, when this member is seized, very much the same curious gyrations as does *Perognathus*. In the former species, however, the vertebræ are not broken, nor indeed is the central axis of the tail severed at all. The skin breaks at some point in its length, and slips off, leaving in one's hands the long tubular sheath which covered the appendage. This process is of such frequent occurrence as to be quite characteristic of these mice, which thus contrast strongly with the various other species of *Peromyscus* that we have observed. In the subspecies of *P. maniculatus*, for example, we have never observed the detachment of any portion of the tail or of the skin, in the course of handling several thousands of these animals. Nor have we observed it in any other genus of California Muridæ.

In *Peromyscus boylei rowleyi* we must again note what seems to be a significant correlation between structure and behavior. The skin of the tail breaks and slips off with remarkable facility.

Likewise, the animal, when seized by this member, makes what appear like vigorous and well-directed efforts to accomplish the severance. In *P. maniculatus*, on the other hand, the skin is far less easily detached, and the animal has scarcely ever been observed to undergo either bodily gyrations or torsion of the tail.

To what extent this detachment of the skin is to be regarded as an "adaptive mechanism," which has arisen because of its utility, we regard it as futile to discuss at present. A single observation may be cited, which, however, can not be regarded as throwing much light on the matter. One specimen of *P. boylei rowleyi* was caught by the tail in a spring mouse-trap, the body being uninjured. Under these conditions, the animal was unable to escape by slipping off the skin and died of shock or exposure. On the other hand, it must be stated that a considerable proportion of the living mice of this species which were lifted by the tail underwent this mutilation, in spite of considerable care being taken to prevent it. What happens to the exposed portion of the tail after the detachment of the skin, has not been observed. It probably dies and falls off.

Scripps Institution for Biological Research,
August 8, 1917.

THE INTERRELATIONSHIP OF THE NUMBER OF STAMENS AND PISTILS IN THE FLOWERS OF *FICARIA*.

J. ARTHUR HARRIS.

I. Introductory Remarks.

A survey of the rapidly increasing literature must convince anyone that the problem of the factors which determine the sex of the organism is one of such complexity that it cannot be solved on the basis of any one kind of material or by any one method of research.

In the flowering plants the same individual may produce both eggs and sperm. The relative numbers of egg and sperm producing organs may vary from individual to individual, or from flower to flower within the individual.

It is reasonable to assume that definite genetic, morphogenetic or physiological factors underlie these variations. Any successful attempt to determine these factors and to measure their influence is just as truly a contribution to the wide problem of the physiology of sex as the more conventional breeding experiments and studies on the morphology of the germ cells.

The purpose of this paper is to point out certain hitherto unrecognized relationships between the number of sporophylls in the flower of the ranunculaceous genus *Ficaria*.

Heretofore those who have investigated the problem of the relationship between the number of stamens and pistils in the flower have been content to merely determine the correlation between the number of the two kinds of spore-bearing organs. Positive correlations of this kind should arise as the resultant of any sets of environmental factors which tend to increase both the number of stamens and the number of pistils in certain of the plants or individual flowers and to limit the number of both of these organs in others. Morphogenetically and physiologically it seems of far greater importance to inquire whether the relative proportion of the two types of spore bearing organs is correlated with the total number of sporophylls, which in lieu of any better character may serve as a measure of the total influence of intrinsic and extrinsic factors influencing degree of development.

Several years ago Professor Pearson and I (Harris, '09) showed that problems of this kind can be approached by determining the correlation between the total organs laid down and the deviation of the number of a particular kind from the probable number on the assumption that the proportion of the particular kind is independent of the total number.

The statistical method may of course be applied to experimental data or to series of determinations made on organisms developing under natural conditions. As yet experimental series are not available.

In a former paper ('16) I showed that in the inflorescences of both *Arisarum vulgare* and *A. proboscidium* there is a significant negative correlation between the total number of flowers and the deviation of the number of staminate flowers from their probable number on the theory of proportional distribution. Thus the male flowers while *absolutely* more numerous in the inflorescences with larger total numbers of flowers are *relatively* less numerous than in the inflorescences with smaller total numbers of flowers. Or, conversely, the larger inflorescences tend to produce *a larger proportion* of pistillate flowers.

In this paper the same analytical methods will be applied to the problem of the relationship of the number of stamens and the number of pistils to the total number of stamens and pistils produced by the flower.

II. Materials.

The materials upon which the coefficients discussed in this paper are based have been tabled and the chief biometric constants deduced by competent statisticians. The special methods upon which the conclusions of this paper are based were not, however, available at the time their calculations were made. The results are, therefore, quite new.

The series employed are the following:

1–2. A series of 283 countings of number of stamens and pistils of *Ficaria verna* from Trogen and another series of 80 countings from Gais, published by Ludwig ('01). Statistical constants for both of these series have been deduced and published by Dr. Alice Lee ('02).

3–4. A series of 268 early and 373 late flowers of *Ficaria ranunculoides* collected by MacLeod ('99) and discussed by W. F. R. Weldon ('01).

5–8. Four series of *Ficaria ranunculoides* collected by Galton, Weldon, Pearson ('03) and others in Italy, Guernsey and England.

III. Presentation of Data.

The means and variabilities of number of stamens and pistils per flower have been given in the papers cited. The only point which requires discussion in this place is the relative variability of the number of the two types of sporophylls. This is shown in Table I.

Table I.

Relative Variabilities in Number of Stamens and Number of Pistils in *Ficaria*.

Series.	Number of Flowers.	Coefficient of Variation for Pistils.	Coefficient of Variation for Stamens.	Differences
Switzerland—				
Trogen, I.	283	23.32	18.68	4.64
Gais, II.	80	23.73	12.18	11.55
Belgium—				
Early, III.	268	23.32	14.07	9.25
Late, IV.	373	27.89	18.46	9.43
Italy, V.	624	22.35	14.12	8.23
Guernsey, VI	520	26.54	17.16	9.38
England—				
Dorset, VII.	505	26.38	16.84	9.54
Surrey, VIII.	500	27.19	17.32	9.87

The number of pistils is consistently more variable than the number of stamens.

Other workers have shown that there is a correlation of medium intensity between the number of stamens and the number of pistils per flower. Their constants, all of which have been rechecked in the course of this work, are shown in Table II. I have also added the linear regression equations showing the rate of increase in mean number of pistils associated with an increase in the number of stamens and the rate of increase in

TABLE II.

CORRELATION BETWEEN NUMBER OF STAMENS AND PISTILS IN *Ficaria* AND REGRESSION EQUATIONS FOR STAMENS AND PISTILS.

Series.	Number of Flowers.	Correlation Stamens and Pistils.	Ratio of Correlation to Probable Error r/E_r.	Regression Line.
Switzerland—				
Trogen..........	283	.530 ± .029	18.27	$S = 11.708 + .645P$ $P = 4.461 + .429S$
Gais.............	80	.388 ± .064	6.06	$S = 19.075 + .262P$ $P = 4.403 + .575S$
Belgium—				
Early............	268	.507 ± .031	16.35	$S = 18.197 + .489P$ $P = 3.427 + .525S$
Late.............	373	.749 ± .015	49.93	$S = 9.006 + .729P$ $P = -1.593 + .769S$
Italy.............	624	.439 ± .022	19.95	$S = 19.313 + .418P$ $P = 5.409 + .460S$
Guernsey..........	520	.534 ± .021	25.43	$S = 18.302 + .404P$ $P = 4.160 + .707S$
England—				
Dorset...........	505	.669 ± .017	39.35	$S = 20.369 + .535P$ $P = -1.333 + .835S$
Surrey...........	500	.660 ± .017	38.82	$S = 19.091 + .588P$ $P = -.860 + .741S$

mean number of stamens associated with an increase in number of pistils per flower.

The regression of the number of stamens on the number of pistils and of the number of pistils on the number of stamens is shown for three of the larger series in Figs. 1–3.

The empirical means for the Italian series, Diagram 1, do not conform very satisfactorily to the lines given by the equations. Better agreements between actual and theoretical means could hardly be found (in series of data no larger than these) than in the Guernsey and Surrey series represented in Figs. 2 and 3.

The main purpose of the present paper is to present the results of the determination of the relationship between the total number of sporophylls and the number of stamens and pistils.

The correlations between the total number of sporophylls and the number of stamens and pistils are shown in Table III.

As is to be expected the correlations between total sporophylls and number of stamens and pistils are high.

The constants showing the relationship between the total number of sporophylls and the deviation of the number of

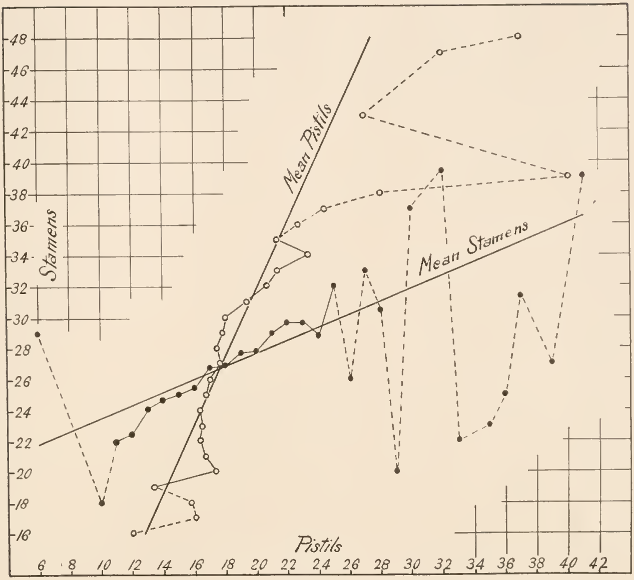

FIG. 1. Average numbers of stamens in flowers with various numbers of pistils and average numbers of pistils in flowers with various numbers of stamens. Empirical and smoothed values. Italian series.

TABLE III.

CORRELATION BETWEEN TOTAL SPOROPHYLLS AND NUMBER OF STAMENS AND PISTILS AND BETWEEN TOTAL SPOROPHYLLS AND DEVIATION OF THE NUMBER OF STAMENS AND PISTILS FROM THEIR PROBABLE VALUE.

Series.	Correlation Between Sporophylls and Stamens.	r_{sz_s}.[1]	r/E_r.	Correlation Between Sporophylls and Pistils.	r_{sz_p}.[2]
I.	.901 ± .008	− .139 ± .039	3.52	.845 ± .012	+.139 ± .039
II.	.755 ± .032	− .548 ± .053	10.37	.896 ± .015	+.548 ± .053
III.	.862 ± .011	− .378 ± .035	10.69	.873 ± .010	+.378 ± .035
IV.	.933 ± .005	− .477 ± .027	17.74	.936 ± .004	+.477 ± .027
V.	.840 ± .008	− .354 ± .024	14.99	.855 ± .007	+.354 ± .024
VI.	.836 ± .009	− .433 ± .024	18.04	.910 ± .005	+.433 ± .024
VII.	.892 ± .027	− .487 ± .023	21.37	.932 ± .004	+.487 ± .023
VIII.	.900 ± .006	− .463 ± .024	19.64	.921 ± .005	+.463 ± .024

[1] Correlation between sporophylls and deviation of stamens from their probable value.

[2] Correlation between sporophylls and deviation of pistils from their probable value.

macro- and the number of microsporophylls from their probable value are the coefficients of critical value. These are also given in Table III. The correlations for stamens and pistils are necessarily equal in magnitude but opposite in sign. They show that the number of pistils is relatively larger in the flowers with larger numbers of sporophylls. The results are consistent in sign throughout. All of the correlations except that for the series from Trogen may be considered certainly significant in comparison with their probable errors.

While the constants for certain of the series differ significantly, the results are (considering the relatively small numbers and the very wide geographical distribution of the material) very consistent. Five of the eight series differ from $r = \pm .50$ by less than twice their probable error. Of the other three series, only Professor Ludwig's Trogen material is very aberrant.

For two of the series I have determined the standard devia-

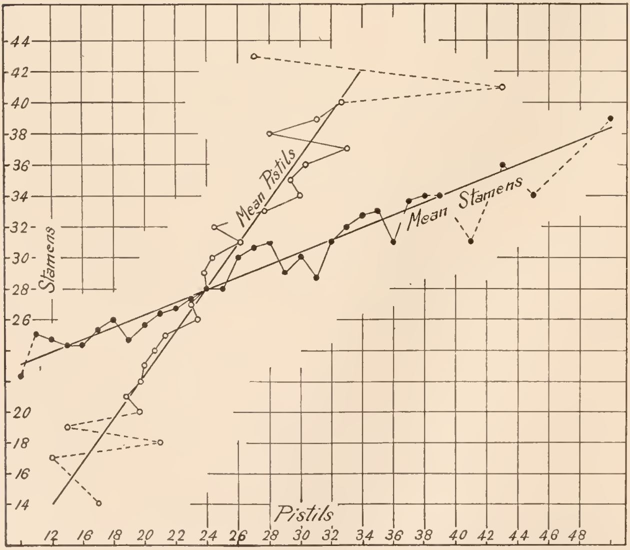

FIG. 2. Empirical means and regression straight lines for regression of stamens on pistils and pistils on stamens. Guernsey series.

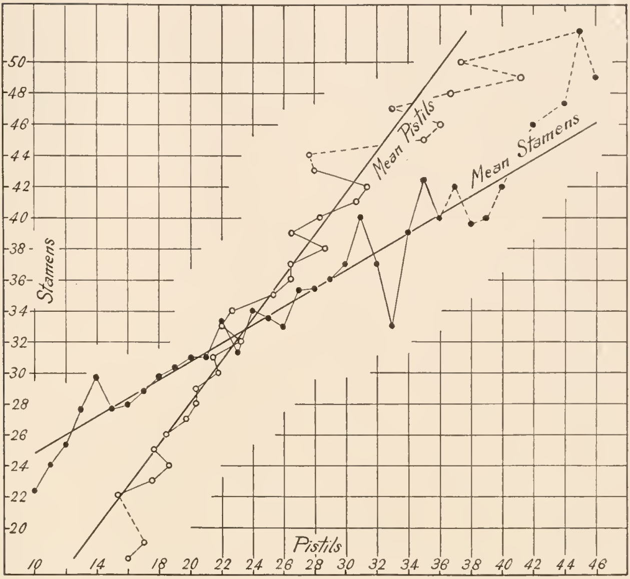

FIG. 3. Explanation as in Figs. 1 and 2. English series.

tion of the deviation of the number of stamens (or pistils) from their probable value by a formula to be published shortly ('17). They are:

For Bordighera, $\sigma_z = 2.1873$

For Surrey, $\sigma_z = 2.8054$

These values make possible the determination of the straight line equations showing the regression of the deviation of the number of stamens and pistils from their probable values upon the total number of sporophylls. They are:

For Bordighera series— $Z_s = +5.2446 - .1181\ S$

$Z_p = -5.2446 + .1181\ S$

For Surrey series— $Z_s = +6.6565 - .1179\ S$

$Z_p = -6.6565 + .1179\ S$

Here Z_s and Z_p denote the deviations of the stamens and pistils from their probable values and S denotes the total number of sporophylls.

The results are represented graphically in Figs. 4 and 5. In the Italian series, flowers with fewer than 36 stamens and pistils are only 29 in number, distributed among flowers with from 28–35 sporophylls per flower. The means at this end of the range cannot, therefore, be expected to show great regularity.

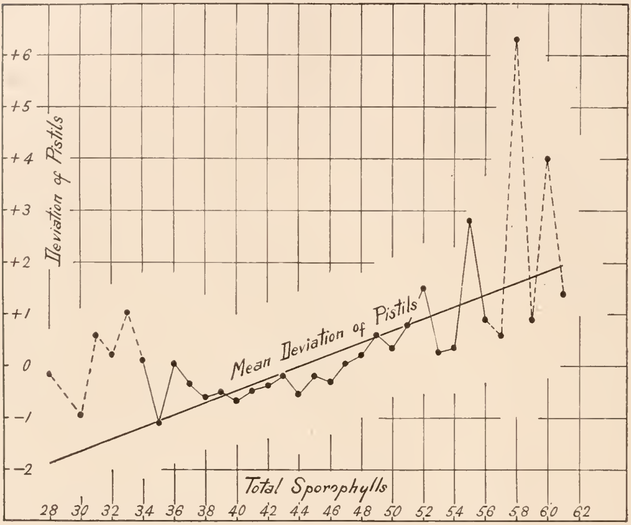

FIG. 4. Regression of the deviation of the total number of pistils from their probable value on the total number of sporophylls. Italian series.

Flowers with more than 61 sporophylls are only 10 in number but are distributed among flowers ranging from 62–85 sporophylls per flower. This portion of the range has not been included in Fig. 4. The relationship is apparently not quite linear.

In the material from Surrey, shown in Fig. 5, the increase in the relative proportion of pistils associated with increase in the total number of stamens and pistils could hardly be better represented than by the slope of the straight line indicated by the equation.

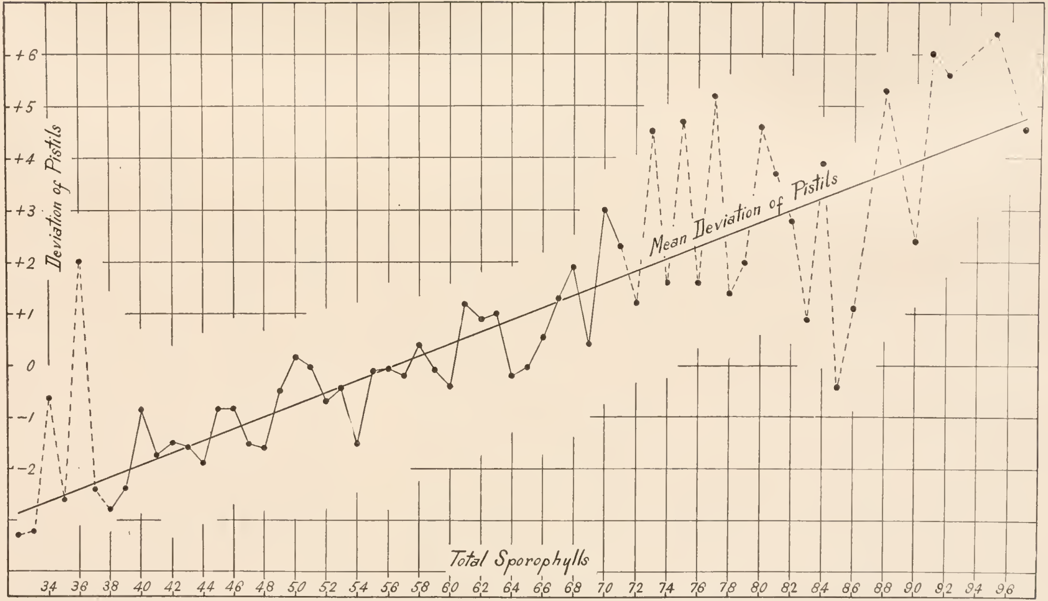

FIG. 5. Regression of the deviation of the total number of pistils from their probable value on the total number of sporophylls. Surrey material.

IV. Recapitulation.

The present study deals with the problem of the relationship between the total number of sporophylls laid down and the relative number of stamens and pistils in the ranunculaceous genus *Ficaria.*

Constants have been deduced for eight series of published data from Italy, Switzerland, Belgium, Guernsey and England not hitherto analyzed by the methods now available.

In flowers with larger numbers of sporophylls the pistils are relatively more numerous than the stamens. The high degree of consistency of the results drawn from such a wide range of habitats indicates that the relationship is one of real morphogenetic significance.

In an earlier paper it has been shown that in *Arisarum* the relative number of pistillate flowers increases as the total number of flowers becomes larger.

It is at least suggestive in relation to the problem of the physiology of sex that in both of these very different forms the number of macrosporophylls becomes relatively larger as the total number of sporophylls increases. The relationships may, however, have an embryological explanation. Only further investigations will justify final conclusions concerning the cause of the relationship demonstrated.

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SOME OBSERVATIONS ON ARTEMIA GRACILIS, THE BRINE SHRIMP OF GREAT SALT LAKE.[1]

ALBERT C. JENSEN.

Very little investigation has been made of the animal life of Great Salt Lake. This is probably because in popular literature we still read that no living thing can exist in the brine of the lake, or because the few forms adapted to such life have failed to interest scientists. So far as the author knows, no attempt has been made to make a complete scientific classification of the lake fauna or to do any extensive research work on it. The brine shrimp, *Artemia gracilis*, one of the lake forms, is a small crustacean found in great abundance.

In referring to the literature on the subject, we find that Captain Bonneville wrote short descriptions of his explorations along the shores of Great Salt Lake, making mention of small animals in the water, probably the brine shrimp, as early as 1831–1833. Fremont also states of rowing to an island in the lake where he found what he called larvæ of insects washed upon the shore in great heaps or windrows three or four feet wide, and many animals living in the water. Verrill described the brine shrimp as *Artemia gracilis* in 1869. Seven years later Dr. Siebold, of Munich, obtained through Dr. Hagen, of Cambridge, Mass., a quantity of dried mud from Great Salt Lake which contained fertile eggs of *Artemia*. These eggs were hatched in artificially prepared brine and shrimps reared therefrom. Siebold succeeded in propagating *Artemia* for several generations and was quite convinced that this crustacean reproduces parthenogenetically as well as sexually. Packard ascribes the abundance of this phyllopod in Great Salt Lake to the absence of enemies. Further, after an examination of specimens he concluded that Verrill's later described species are merely different stages of the same species and should be included under the first described form, *Artemia gracilis*. As late as 1889 David Starr Jordan stated that the lake water was so salty that no life could exist in it except the brine shrimp. Gilbert suggests that the presence of *Artemia* is no mystery for its ancestors may have lived in the fresh water of the basin and have transformed with the increasing saltiness of the lake. Talmage observed that the little phyllopod existed in great numbers in the lake, especially between the months of May and October. He states having collected them in the midst of winter when the temperature was far below the freezing point of fresh water, and from the evaporating ponds of the salt works, where the brine was near the point of saturation. He was able to keep them alive for several days in various dilutions of lakewater, and noted that they would even live for some time in distilled water. He further observed that repeated washings in fresh water for five minutes removed the brine so completely that salt had to be added to make them a palatable food.

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[1] Contribution from the Zoölogical Laboratory of the University of Utah.

The adult *Artemia*, Plate I., has a small head carrying a single black median eye (ocellus), a pair of large stalked compound eyes, two pairs of antennæ, one pair of mandibles and a pair of maxillæ. The second pair of antennæ of the female are short and pointed, while those of the male are large and broad, Plate II. The thorax bears eleven pairs of legs which are leaf-like in structure and serve as swimming organs and for the attachment of the respiratory organs. The abdomen is long, composed of eight segments without appendages. A number of bristles are to be found on the terminal segment. The ovisac is roughly bottle-shaped, being rather short and broad and attached to the first and second abdominal segments.

The œsophagus is short and leads to the stomach, which is situated in the head. The stomach is divided into two lobes, each of which receives a duct from the liver. The liver is two-lobed and lies in the head anterior to the stomach. The intestine is a straight tube. The division extending through the thorax is twice as large as that passing through the abdomen. The intestine includes a well-marked rectum, which is provided with constricting circular muscles. The ovaries are located in the first and second abdominal segments ventral to the intestine. The testes are located in the same relative position. The heart is a long tube beginning just posterior to the mandibles and terminating in the anterior part of the last abdominal segment. It has a distinct valve at the posterior end and apparently a pair of valves for each segment, Fig. 1. The blood enters the heart through these side ostea and is forced out through the ends of the heart. The central nervous system consists of a two-lobed supraœsophageal ganglion in front of the œsophagus, and just behind the œsophagus, between the digestive canal and the floor of the body, is the subœsophageal ganglion, the first of a chain that extends through the body along the ventral side. Nerve fibers extend from these ganglia to the body and appendages.

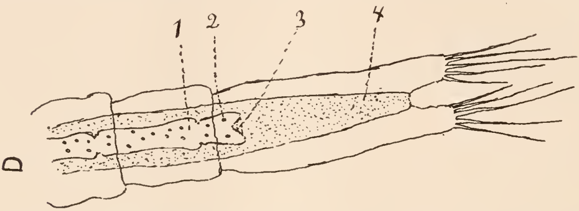

FIG. 1. Last three abdominal segments. 1, Heart; 2, blood corpuscles; 3, terminal valve; 4, intestine.

The sexes are separate in *Artemia*. The female is 10 to 12 mm. long, while the male has a length of 8 to 10 mm. Reproduction is accomplished by means of eggs that may or may not be fertilized.

In copulating the male claspers are placed around the body of the female just in front of the ovisac and thus attached the pair may swim about for several days, as many as five having been observed. At intervals the male's abdomen may be seen to bend so that the genital appendages come into contact with the oviduct. Frequently copulation takes place before the ovisac begins to show a brown color, due to the color of the eggs, and in such cases the eggs are retained in the ovisac for as long as three days after copulation has ceased. In other instances copulation does not take place until the eggs are brown in the ovisac and often these are deposited and even hatched while copulation is still in progress. It is presumed that such a copulation is too late to fertilize the eggs for they have passed from the ovaries into the ovisac and are covered with a brown shell and are ready to be deposited. These eggs, deposited during April and May, hatch within twenty-four hours after being laid.

A comparison of these newly deposited eggs with those collected during the early spring showed no particular difference, except that none of the new eggs were cup-shaped as are many of the winter eggs, Plate III., *A*. Examination of a number of females showed the number of eggs to vary between sixteen and one hundred and twenty. The females deposit their eggs while swimming.

Eggs were collected March 8 and kept in the laboratory in normal lake water for twenty-one days. The room temperature apparently had little effect upon the development since nauplii were collected in the lake three days after individuals were observed in the aquarium.

The embryo transforms into a nauplius much unlike the adult, Fig. *E*, Plate III. It has a blood-red color, a median eye (ocellus) and three pairs of appendages, the first of which develop into the first pair of antennæ, the second pair are much larger and serve as swimming organs, while the third pair become the mandibles. After a few days the second pair, in females, become shorter, less movable, lose their bristled margins, and are transformed into small scarcely movable processes, the second antennæ, Plate II., *B*. In the males they also form the second antennæ which develop into disproportionately large claspers with broad lobes, Plate II., *A*, and are used as catching and clasping organs. Along with the early development of these are signs of segmentation which is followed by the appearance of the thoracic appendages one pair after another until all eleven pairs have been laid down. The stalked compound eyes appear about the same time as the first thoracic appendages. Within eighteen to twenty-one days the animals reach sexual maturity, shown by copulation. Sexual differences become apparent when the animals are a little more than half grown. The males are stronger and more active than the females.

Siebold's conclusion that *Artemia* may reproduce parthenogenetically is verified by my observations. Water was taken from the lake before nauplii appeared there, and precautions taken to eliminate all eggs. Undeveloped females were placed in this water, where they developed normally and deposited eggs similar in appearance to fertilized ones. These eggs produced males and females, which developed normally and as far as could be observed could not be distinguished from those coming from fertilized eggs. A large proportion of the hatch was females. Both methods are evidently used during the summer months.

According to my records all of the brine shrimps die during the winter months, probably due to the lowered temperature. These observations do not harmonize with those of Talmage, who says: "I have taken them in the midst of winter, when the temperature of the water was far below the freezing point."

Statements made by Gilbert that the ancestors of *Artemia* may have lived in the fresh water of the basin, led to an experiment to determine the effect of different concentrations of salt water upon this animal. By using distilled water and the process of evaporation, solutions were prepared having a specific gravity as follows:

I.	II.	III.	IV.	V.	VI.	VII.	VIII	IX.
Dis. water					Normal lake water	Evaporated to	Evaporated to	Evap. to saturated point
1.000	1.027	1.044	1.064	1.085	1.109	1.130	1.711	1.179[1]

On March 28 eggs were put into the above solutions. March 30, 44 hours later, nauplii had emerged and were swimming about in solutions 1, 2 and 3 and in 4 they were emerging from the eggs. In the latter I observed the ocellus, also one or two pairs of appendages that were moving as if trying to free the body from the shell. By April 10 nauplii were emerging in solution 5 and not until April 13 did the eggs in solution 6 begin to hatch. By this date nauplii were also emerging from eggs in the lake. On May 5 a few nauplii appeared in solution 7, but in solutions 8 and 9 no nauplii had emerged. To test if the eggs in these last two solutions were fertile, a few were put into a less dense solution where they hatched readily. In solution 1 eggs hatched readily in twenty-four hours, but the nauplii lived only from one to three days. However transferring nauplii from 1 to solutions 2 to 6 inclusive brought good results. In solutions 2 to 6 the nauplii, hatched in the order stated above, lived and developed rapidly to maturity. Young and adult *Artemia* transferred to solutions 8 and 9 from less dense solutions died within five to ten hours, the young dying first. Those put into solution 7 developed slowly, while those placed into solutions 2 to 6 developed quite normally. Solutions 3, 4 and 5 however seemed to be the most favorable for the growth of *Artemia*. They would also develop normally when transferred among any of the solutions 2 to 6 inclusive.

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[1] The specific gravities herein given are the uncorrected hydrometer readings.

Artemia is often found in great numbers. Fremont in writing of his explorations of the lake speaks of windrows of the brine shrimp and the pupa cases of insects washed upon shore. Talmage states having seen the surface of the water tinted by them and also of having gathered with a tow-net a quart within a few minutes. Indians inhabiting this region used to collect large quantities of the crustacean along with insect larvæ which they dried and used as food.

The brine shrimp is a vegetable feeder. Near shore may be seen many masses of an alga, *Aphanothece*, and it is along with this that *Artemia* is most abundant. This alga and its gelatinous secretion forms the principal food for the phyllopods as shown by the fact that they may be seen feeding upon it, and it can also be demonstrated in their digestive tracts. In fact this alga seems to be their only food.

Artemia gracilis is chiefly a littoral form, since in this zone its food is most abundant. However these crustaceans have been observed by Talmage in great swarms far out in the lake where they were carried by winds unless they have swarming or migratory habits.

There is a very disagreeable odor encountered upon approaching the shores of Great Salt Lake at certain places. This stench has been attributed to the decomposition of the brine shrimp. The odor is at times very offensive, which, by some, is thought to be dangerous, and as a result the summer resorts of the lake are not visited by many people who would otherwise enjoy them.

The stench is due primarily to decaying vegetable matter. Vessels placed in the laboratory containing plant material from the lake gave off the characteristic lake odor. Further the odor was present at the lake as early as the last of March, before the appearance of *Artemia*. Again the proportion of the phyllopods to the plant material is so small as to make the brine shrimp an insignificant factor in the odor production.

CONCLUSIONS.

Winter eggs of *Artemia* kept in normal lake water at room temperature (about 20 degrees centigrade) for a period of two months did not hatch earlier than those in the lake.

The embryo of *Artemia* develops into a free-swimming nauplius, much unlike the adult, which passes through a series of stages before reaching maturity.

Artemia reproduces by means of fertilized eggs and also parthenogenetically.

Eggs will hatch during the winter as well as spring and summer in dilute lake water or in fresh water at a temperature as low as 20 degrees centigrade. My experiments show also that eggs will not hatch in solutions concentrated to near the saturation point.

Artemia passes the winter months in the egg stage.

Artemia will not live in fresh water, but will in a dilution of the lake water having a specific gravity of 1.027.

Lake water diluted to a specific gravity of 1.044 to 1.027 is most favorable for the development of *Artemia*.

I take this opportunity to heartily thank Dr. Newton Miller for his assistance in this work.

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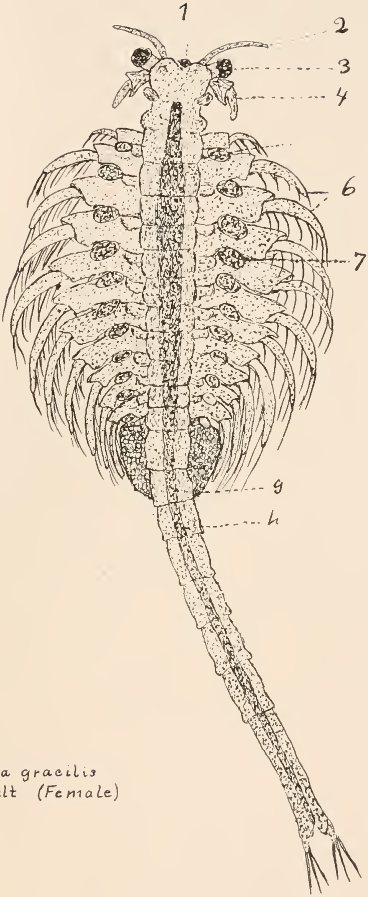
EXPLANATION OF PLATES.

All figures drawn with the camera lucida.

PLATE I.

Adult female *Artemia gracilis*.



Artemia gracilis
Adult (Female)

ALBERT C. JENSEN.

PLATE II.

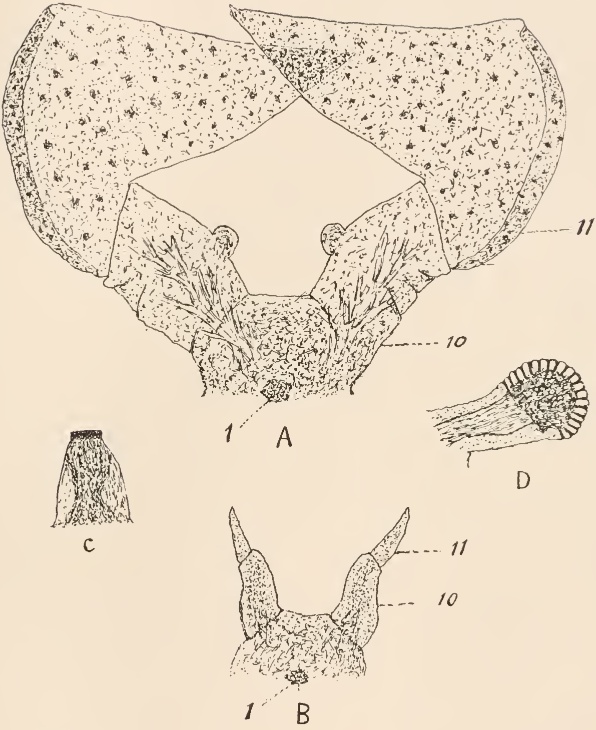
A. Head of male showing first pair of antennæ.

B. Head of female showing first pair of antennæ.

C. Mandible.

D. Compound eye.

Figures of this plate drawn on the same scale.


11
10
1
A
D
C
11
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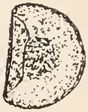
1
B

ALBERT C JENSEN.

PLATE III.

A. Cup-shaped egg.
B. A spherical egg.
C and *D*. Nauplii emerging from eggs.
E. Early nauplius stage.



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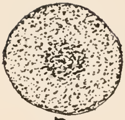

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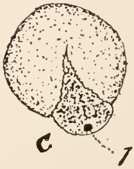

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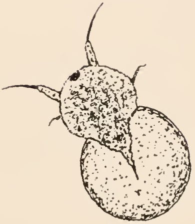

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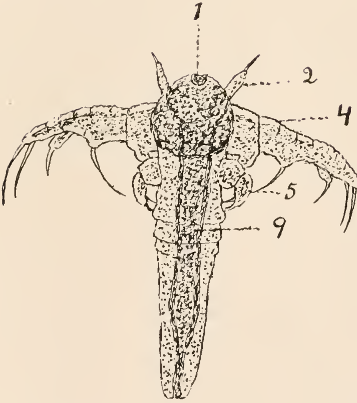

E

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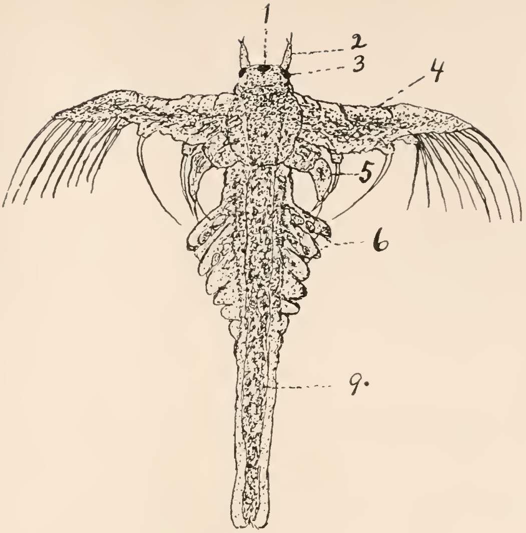
PLATE IV.

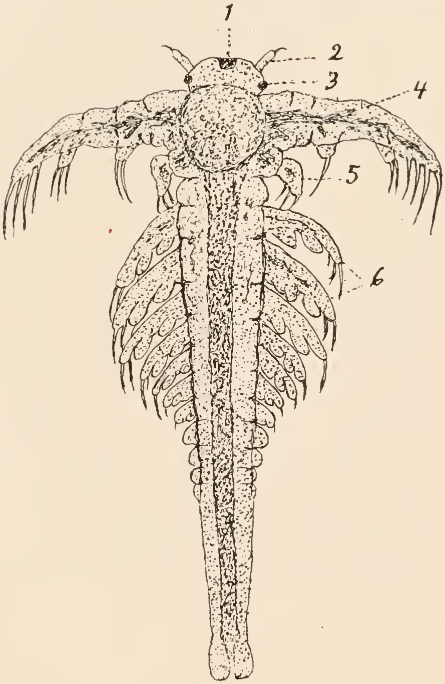
Later nauplius stages.

Figures of plates III. and IV. drawn on the same scale.

Notations.

1. Ocellus.
2. First antenna.
3. Compound eye.
4. Second antenna.
5. Mandible.
6. Thoracic appendages.
7. Gills.
8. Ovisac.
9. Intestine.
10. Basal segment of the first antenna.
11. Distal segment of the first antenna.





ALBERT C. JENSEN.

THE RELATIONS BETWEEN THE INTERSTITIAL GLAND OF THE TESTICLE, SEMINIFEROUS TUBULES AND THE SECONDARY SEXUAL CHARACTERS.[1]

LEO LOEB.

The following observations may prove of interest in the analysis of the relations between male gonads and secondary sexual characters.

While, as is often the case, an experiment of nature, unaided by experiments in the laboratory, is unable to give a definite answer to the questions which it raises, yet it would at present be impossible to duplicate at will in the laboratory an experiment such as we observed and it is, therefore, desirable that it should be recorded.

Our observations were as follows:

Guinea pig No. 1523 had been sent to our laboratory by an animal breeder as a female that had apparently been in heat, but in some respects behaved abnormally. The report of the breeder was as follows:

"The female through her actions gave every sign of being in heat; but upon examining her, she did not look to me as if she really was in heat. I had her with two other females and she would mount them just the same as a male guinea pig would do when in the act of copulation and she would also allow the other females to act in the same way. She would stand in this position, meanwhile expanding the vagina, which generally is a sure sign of their being in heat. I would say that she is with young; at least she should be pregnant, because she had been with a male for some time. Expect some young at any time."

The breeder made his observations July 8, 1917. On July 12, 1917, the animal, which weighed 615 grams, was prepared for operation. Under ether anesthesia lumbar incisions were made, in order to expose the ovaries. Typical ovaries were, however, not found. Instead, we encountered near the place where the ovaries usually are situated, but perhaps a little farther down, somewhat large and round, rather soft bodies, each about the size of a pea, surrounded by much fat tissue. On cutting through the center the color was found to be slightly yellowish-brown. From here some fibrous bands extended downward in the direction in which the uterine horns are usually situated. An organ that resembled the uterus was not found. The vagina was also lacking. Neither was a penis, vas deferens or a descended testicle visible.

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[1] From the Department of Comparative Pathology, Wshhington University Medical School, St. Louis, Mo.

The brownish red bodies as well as pieces of the fibrous bands were fixed in Zenker. Both of the bodies were completely cut into serial sections. Tissue from the places where the mammary glands are usually situated was also fixed and sectioned.

Microscopic examination:

1. The round bodies situated below the kidney were seen, microscopically, to consist of testicle tissue in which there was an extraordinarily marked development of interstitial gland. The testicle tubules were lined by one layer of epithelial cells, the outline of which was not very definite. Towards the center of the tubules they formed a network of fine fibrils; a sharply defined lumen therefore did not exist in the tubules. The cells had either a cuboidal or cylindrical form as far as their indefinite outline permitted of such a characterization. In some cases the whole protoplasm of the cell was drawn towards the center of the tubule in a tail-like structure. The nucleus was situated in the center of the cell and was very characteristic. It represented a clear vesicle in which one deep-staining large nucleolus was visible. Occasionally the nucleolus divided into two parts. In various places the tubules were lined with several cell rows. Sometimes the cytoplasm and the nucleus of one cell swelled, and such a cell encroached upon the territory of the neighboring cell, lying more towards the center of the tubule, pressing against it; and thus one cell surrounded, crescent like, the neighboring cell. Occasionally some cells degenerated, the nucleolus persisting longest, while the rest of the nucleus had already disappeared. Mitoses were seen quite frequently in these tubule cells and monasters as well as diasters

were found. The tubules were enveloped by a circular layer of flat connective tissue cells, which were drawn out into long fibers with flat nuclei. Each tubule was surrounded by several, usually two to five, layers of interstitial cells. Each tubule with the surrounding concentrically arranged interstitial cells formed a group which was often quite sharply separated from neighboring similar groups through lymph vessels or lymph-spaces and blood vessels and accompanying connective tissue strands. In certain places larger blood vessels accompanied by lymph vessels were found in the tissue. Blood capillaries were numerous in certain places. Occasionally a tubule was found adjoining the connective tissue directly, without being separated from the latter by interstitial cells. The character of the interstitial cells varied somewhat in different parts of the organ. Usually they were large polygonal, partly rounded off or oval cells with a large vesicular nucleus, in which several small nucleoli were generally seen, instead of the one clear nucleus which was so characteristic of the tubule cells. The nucleus in the interstitial cell was not so light and transparent as in the tubule cell. The sizes of the interstitial cells varied. The cytoplasm became finely vacuolar, at first in the periphery. In the center there was still a solid material staining red with eosin. Later the whole cytoplasm became finely vacuolar. This vacuolization was usually accompanied by a considerable increase in size of the cells. In some cases the vacuoles enlarged still more, the walls separating neighboring vacuoles disappeared and irregular cavities became visible in the cells. In those cases the nucleus became irregular and shrank, and when this last stage had been reached, the cell was evidently degenerating. Many finely vacuolar enlarged cells appeared otherwise normal and viable. These finely vacuolar, swollen cells occurred especially around the lymph vessels, or perhaps also around certain blood vessels. We must assume that substances reaching the cells from the circulation were deposited within the cells and caused the vacuolization, the nearness to the source of this substance determining the frequency and intensity with which these changes took place. Under the same conditions the accompanying fibrous tissue became edematous. Apparently the vacuolization of the interstitial cells and the edematous state of the stroma are analogous phenomena. We were only rarely able to find mitoses in the interstitial cells. In this respect the latter differed from the tubule cells in which mitoses were quite frequent. There could be found at one end of each testicle a system of branching epithelial ducts, with a narrow lumen. They were lined by a layer of small and densely packed, usually cuboidal or flat epithelial cells, with nuclei which filled the greater part of the cells. In these cells either one or several nucleoli were found. If one nucleolus was present the nucleus resembled that of the tubule cells, except that it was smaller. At other places the lining cells and nucleus were low cylindrical. In the lumen of these ducts not infrequently some invaginations of the epithelium were found. On the one side the ducts formed a union with the tubule of the testicle. Between the two we found transitional structures consisting in part of tubular and in part of duct epithelium. At the other end the ducts entered the fat tissue which surrounded the testicle and made connection with some larger ducts. One change that took place in a number of tubules needs special mention: A few or many, at some places even the majority of the tubule cells underwent certain changes which made them very similar to interstitial cells. They enlarged, became polygonal or somewhat round, and their cytoplasm stained more strongly with eosin. The cytoplasm became finely vacuolar, at first the periphery and later the greater part of the cell undergoing this change. At the same time the nucleoli divided into several particles which were dispersed in the nucleus. The nucleus became a round vesicle which had lost the transparency so characteristic of the nucleus of the tubule cells. Accompanying this change the layer of flat connective tissue cells which separated tubules and interstitial cells disappeared and at various places interstitial cells adjoined directly the tubule cells, both kinds having become very similar. Such changes seemed to be frequent especially in places where the interstitial cells were swollen and had become finely vacuolar. Three interpretations of this condition suggest themselves:

1. As a result of their enlargement the interstitial cells push into the tubules, and thus the appearance within the tubules of typical tubule cells adjoining directly cells resembling interstitial

cells might be explained. At the same time the enlarged interstitial cells exerted a pressure on the tubule cells which made them crowd closer together than usual. This interpretation is not admissible, because the cells within the tubule began to swell at a time when the connective tissue layer separating tubule and interstitial tissue was not yet broken through. At first the enlarging cells still possessed the nucleus characteristic of the tubule cells and only gradually the character of the nucleus changed concomitantly with the later stages of the transformation.

2. It might be assumed that an actual transformation of tubule tissue into interstitial tissue took place, and that the gland-like interstitial tissue was merely further differentiated tubular gland tissue. Many pictures seemed to suggest such an interpretation. However, the fact that in many other places, especially where the interstitial cells were still solid and smaller, and, therefore, younger, the line of demarkation between tubule and interstitial tissue throughout was very sharp made, after all, this interpretation improbable. Furthermore, it would be contradicted by what is known of the origin of the interstitial cells.

3. It is most probable that the same factors which produce the changes in the interstitial cells, at the same time called forth similar changes in the tubule cells. Probably certain substances furnished by the lymph or blood vessels were simultaneously responsible for both sets of changes. In favor of this interpretation might be cited the fact that we did not usually find in the tubules that extreme vacuolization, which was present in some of the interstitial cells. We are, therefore, inclined to adopt the third interpretation.

The round bodies which we found near the place where normally the ovaries are situated therefore represent undescended testicles. There is a system of ducts leading from the testicle tubules to the surrounding fat tissue. The tubules are relatively simple structures in which spermatogenesis does not take place. The epithelium lining them corresponds to the sustentacular cells of the normal testis. There is present an extremely marked development of the interstitial cells. The structure of this testicle differs, therefore, markedly from the normal testicle of the guinea pig which consists principally of tubules with several layers of epithelium which are in the process of spermatogenesis. Between these tubules there is very little stroma with blood vessels and a very small number of polygonal cells.

Sections through the tissue, taken from the place where normally the uterus is situated, show fibrillar connective tissue in which are situated nests composed of rather large polygonal, cylindrical or ellipitical cells, the protoplasm of which stains well with eosin. The nuclei of these cells are round vesicle with chromatic particles distributed rather diffusely. These cells form the matrix of the neighboring fibrillar connective tissue. Through rarefaction of the cytoplasm and through a condensation, backing together of the nuclear chromatin these cells are transformed into the surrounding connective tissue. We also studied in microscopic sections the fat tissue which filled the space between the undescended testicle and the kidney, without finding any trace of further testicular or of ovarian tissue.

Mammary Gland.—The nipples of the guinea pig were distinct and we cut out pieces from the tissue where normally the mammary gland is situated. In the male a few scattered ducts lined by epithelium in which mitoses do not usually occur represent the mammary gland.[1] In our case, a very much furthergoing development of the mammary gland was found on microscopical examination. Sections through one of the two mammary glands showed solid tissue consisting of a large number of lobules of mammary gland tissue, joined together by thin strands of fibrous tissue. The whole gland was surrounded by fat. Each lobule consisted of acini lined by cuboidal epithelium of medium size. The nuclei were vesicular and relatively large. The lumen of the acini varied in size and often contained some colloid material or desquamated cells. Occasionally an acinus cell was found to be vacuolar. In the stroma between the acini there were numerous small connective tissue cells. At some places, where the interstices between the acini were somewhat wider and the stroma was slightly edematous, polynuclear leucocytes staining deeply with eosin were found. The largest ducts were as usual surrounded by denser fibrous tissue. At one pole of the gland a system of ducts and acini lined by higher and larger epithelium extended into the surrounding fat tissue. The lumen of these ducts and acini was wider. Mitoses could not be seen in the mammary gland. The mammary gland of the other side, which was also surrounded by fat, was smaller, at least the amount of gland tissue found on sections, which had been through different parts of the piece, was less; but it still surpassed considerably that found in normal animals. Furthermore, the acini were composed of high cylindrical cells, surrounding a rather large lumen. There were a few desquamated cells in the gland lumina, the acini being arranged around the larger ducts. Here and there mitoses were seen in the acini. The stroma between the acini was loose in texture and contained a number of fibroblasts.

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[1] In the ducts of the mammary gland of a young male guinea pig, about 30 days old, we encountered however one mitosis.

Discussion.—The following are the chief points of interest in these observations:

1. We have in this case apparently to deal with an undescended testicle in an adult guinea pig. Such testicles have been observed in man and several species of animal, especially in the horse and pig. This case is an example of the same condition in the rodentia. Common to all cases which have been so far described is an imperfect development of the seminiferous tubules and a hypertrophy or a hyperplasia of the interstitial cells of the testicle. As to the literature concerning such cases we may refer especially to Bouin and Ancel (1) Tandler and Grosz (2), and Whitehead (3). Both of the features we mentioned are present in a marked degree in our case. Spermatogonia are completely absent; the tubules consist entirely of Sertoli cells. The retention of the testicle acted in a way comparable to the ligation of the vas deferens which brings about similar results which are, however, usually not quite so far going. In addition, it is very probable that the condition which prevented the normal descent of the testicle was directly responsible for the lack of development of spermatogonia. As to the overdevelopment of the interstitial gland, in those cases, we may conclude that it stands in some causal relation and is subsequent to the underdevelopment of the seminiferous tubules. In various other conditions in which atrophy

or underdevelopment of the tubules has been observed, or produced experimentally (ligation of vas deferens, Roentgen ray injury of the testicle (4) (5) and certain diseases) a hypertrophy or a hyperplastic condition of the interstitial gland has been noted. Herxheimer and Hoffman (5) suggest that this hyperplasia is comparable to the substitutive growth of connective tissue which takes place whenever parenchymatous elements are destroyed. This explanation, however, does not seem to take into account the principal variations which have been observed in the condition of the interstitial gland. Bouin and Ancel (1) have shown that in the horse the interstitial gland is present in the fetus; it degenerates in the immature animal and begins anew to develop at the time of puberty. In the sexually mature animal there seems to exist a certain antagonism between the activity of the seminiferous tubules and the interstitial gland. The interstitial gland becomes hyperplastic in cases in which the tubules decrease in activity and vice versa. Under the conditions of tubular atrophy or degeneration which we named above the interstitial gland hypertrophies, and according to Tandler and Grosz in the mole, in which sexual activity follows a yearly cycle, the interstitial gland shows the greatest development at a time when the seminiferous tubules are least active. In hibernating animals the interstitial gland decreases at the period of general metabolic inactivity (von Hansemann); similarly in cases of very pronounced general undernourishment, or of very marked degenerative processes in the testicle the interstitial gland suffers and may disappear. Some observations indicate, furthermore, that under certain conditions extirpation of one testicle may lead to a hypertrophy of the interstitial gland in the second one. All these facts can evidently not be explained otherwise than by assuming that there are several factors active and that chemical and not merely mechanical substitutive factors play a rôle in the regulation of the growth of the interstitial gland. It seems to be a general function of gland and other epithelial cells to stimulate through their activity the surrounding stroma. We have described such an occurrence especially in connection with the cyclic growth of the mammary gland, but it seems to be a phenomenon of general significance. In a similar way we may assume that growth processes in the seminiferous tubules stimulate the activity of the connective tissue cells and those specialized connective tissue cells which have the potentiality of becoming transformed into interstitial gland. This would explain the development of the interstitial gland during embryonic development and at the time of puberty. We might designate this as the concomitant growth of the interstitial gland. In order to explain the second kind of growth in the interstitial gland, which we might call the "alternative growth," we must assume that in a certain way the activity of the sexually active seminiferous tubules exerts an inhibiting effect on the growth of the interstitial gland and that a diminution of this inhibiting effect leads to the "alternative" growth of the interstitial gland. An analogous condition is found in the rabbit ovary in which the atresia of certain follicles leads to a secondary gland-like development of the theca interna. How far such an equilibrized condition is maintained through chemical, how far through finely adjusted mechanical factors, we do not know at present. However, in the somewhat analogous case of the transformation of the connective tissue cells of the uterine mucosa into decidua, we have analyzed the underlying factors experimentally and found that a combination of specific chemical and mechanical factors, which latter are likewise to a certain extent specific, is responsible for this change (6).

In addition, the interstitial cells of the testicle are accessible to that chemical stimulation which is implied in compensatory hypertrophy and they are furthermore affected by lack of common foodstuffs.

2. The specific effects exerted by the interstitial cells have been ascribed by Bouin and Ancel to certain products formed within the cells which microchemically behave like fats or lipoids. We found, in our case, reasons for assuming that drop-like substances were furnished the interstitial cells from the surrounding lymphatics and perhaps also from the blood capillaries. How far the substances taken up from the circulation and perhaps modified within the cells are identical with the substances described by Bouin and Ancel and Whitehead, we cannot at present decide.

3. Various writers, especially Bouin and Ancel, concluded that the interstitial cells of the testicle are responsible for the development of sexual desire in the male. Our observations seem to confirm this view. The complete absence of spermatogonia in the tubules, together with the marked development of the interstitial gland was accompanied by the presence of marked manifestations of sexual instinct.

4. Bouin and Ancel, Steinach (7, 8) and Tandler and Grosz not only ascribe to the interstitial gland the function to cause chemically those nervous changes which find expression as sexual desire, but the additional task of enhancing the development of the male sexual characters, of allowing the full development of prostate, penis, seminal vesicle, and of suppressing the development of the female secondary characters. Our observations apparently contradict these conclusions. In our case, the very marked development of the interstitial gland was accompanied by the absence of penis, seminal vesicles (and presumably of prostate), and on the other hand was associated with a very marked development of the mammary gland. The latter resembled a proliferating breast, a "proliferating gland" such as Dr. Hesselberg and the writer found it in certain stages of the sexual cycle in the female guinea pig (9). Our observations prove that the presence of a very strongly developed interstitial gland is perfectly compatible with the existence of certain female, and with the absence of certain male secondary sexual characters. A priori two interpretations of this fact are possible:

(*a*) The interstitial gland of the testicle has not a specific function in the sense assumed especially by Bouin and Ancel and Steinach. (*b*) The interstitial gland is specifically "male enhancing" in its function, but the end effect does not only depend upon this specific action of the interstitial gland, but also, and primarily so, on the system on which it acts. We have reason for assuming that in a certain respect an individual can be compared to a more or less sensitive balance in his or her sexual potentialities and that different individuals of a certain species differ in the resting point of their sexual equilibrium (10, 11, 12, 13, 14). Without the interference of hormones which are produced by the gonads, the point of equilibrium in some individuals would be neutral; in others it would be deviating more or less in the direction toward the male; in still others toward the female side. On the whole the male would usually deviate to the male and females to the female side. However, differences exist in this respect in different classes and species of animals and also in individuals of the same species. There are, furthermore, differences in the relative degree of stability of this point of equilibrium. In insects it is apparently very stable and little or not at all influenced by hormones of gonads; in mammals the equilibrium is labile and readily influenced by hormones of the gonads. In mammals, the male hormones can be compared to weights added to the male side of the scales and female hormones influence the scales in the opposite direction. It seems probable that in our case we had to deal with an individual with imperfect male gonads which represented a system with a very slight tendency towards the male side and with a relatively strong tendency toward the female side. In such an individual with tendency toward female secondary sexual characters even a strong male hormone is not able to prevent the growth of the mammary gland, and this female tendency is probably in some way connected with the suppression of male secondary characters during embryonic development, and perhaps also directly or indirectly with the failure of the testicle to descend to its proper place and develop perfectly. In such a relatively stable system tending toward femaleness in its secondary characters, even a specific male hormone, such as is supplied by the interstitial gland of the testicle, has little chance to become effective. Whether, under certain conditions, the hormone given off by the interstitial gland of the testicle might in addition be able to promote growth of the mammary gland, cannot be decided on the strength of the evidence which we have at present.

Summary.

1. It is shown that in a guinea pig with undescended testicle, in which spermatogonia were lacking, and the interstitial gland was hypertrophic, sexual desire was strongly developed.

2. An attempt is made to analyze the various factors that determine the growth of the interstitial gland in the testicle.

3. It is shown that, notwithstanding the presence of a hypertrophic interstitial gland, there was an absence of male secondary sexual characters and that the mammary gland had a female character. A provisional interpretation of these facts is given on the basis of our present knowledge of sex determination.

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ADDITIONAL NOTE

The foregoing paper was written without the knowledge of two quite recent, very important publications by F. R. Lillie and C. L. Chapin, which may have some bearing on our observations, and which it will therefore be necessary to briefly discuss from

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[1] F. R. Lillie, *Journal Exper. Zoölogy*, 1917, XXIII., 371. C. L. Chapin, *ibid.* 453. I am much indebted to Prof. Lillie for calling my attention to these papers,

this point of view. In the first of these two papers Lillie gives a detailed account of the anatomy of the internal and external genital organs in freemartins in various stages of embryonic development and of his interpretation of the cause of the abnormalities and in the second paper Chapin describes the microscopical findings in these cases. It follows from Lillie's observations and analysis that the sterile freemartin represents an individual which was originally a female, but in which under the influence of hormones carried into her circulation from the chorionic vessels of a male twin, the female characteristics were more or less suppressed and male characters acquired. This not only applies to the secondary sexual characters, but even to the structure of the gonads. As Chapin has shown, the development of the ovarian cortex and of ovarian follicles is more or less interfered with or may be altogether suppressed, and the medullary sex cords develop often in a way similar to the seminiferous tubules, although spermatocytes or spermatozoa to not seem to be produced. The development of the Müllerian ducts is likewise interfered with and seminal vesicles may develop instead. The external genitalia and the mammary gland are least affected by the male forming substances, which according to Lillie originated in the testicle of the male twin and were from here transmitted to the female twin; these latter organs remain usually female in character.

These important findings may have some bearing on the interpretation of our observation. There exist undoubtedly marked similarities between the condition found in the freemartins and in the condition of the genital organs of the guinea pig which we examined. However, while Lillie and Chapin examined embryological material, we have to deal with an adult animal.[1] In our case we observed a gonad which shows the characteristics found in testicles under various pathological conditions. Similar changes have been produced in testicles experimentally under the influence of Roentgen rays. The tubules, the character of the cells composing them and particularly of the nuclei closely resemble structures which we found after transplantation of testicle and epididymis in the guinea pig. The interstitial cells are developed to an extraordinary extent. The organs were cut in complete serial sections and no trace of ovarian structure was found. From the descriptions given by Miss Chapin, I should judge that a similar condition has not been observed in the fetal gonads of the freemartins. In the guinea pig, uterus and vagina as well as vas deferens, seminal vesicles and penis were absent. While I was unable to find a description of the histology of the mammary gland in freemartins, we observed in our case a typical female mammary gland with the added peculiarity that the mammary glands of both sides were unequal.

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[1] Microscopic sections through the gonads of an older freemartin from the material of Dr. Lillie show a great similarity with our sections through the gonads of the guinea pig.

The question arises, whether the guinea pig which we observed was an analogue to the freemartin in cattle. Was this animal originally the twin sister of a brother, under whose influence the observed deficiencies were produced? While we cannot definitely exclude such a possibility we must consider the fact that neither ourselves nor anyone else has so far observed twins with a common chorion and deficiencies in the development of the sexual organs in rodents. Lillie himself points out the great improbability of such an occurrence in animals in which the ova enter the uterine wall at an early date and in which the uterine horns are separated. On the other hand, we accidentally observed in several cases absence of one uterine horn in guinea pigs. Malformations of the sexual organs do therefore occur. It is very probable that similar malformations may be due to different interferences in the embryo, just as we know that cyclopic conditions of the eye can be produced in different ways. We interpreted in our paper the conditions which we found in the guinea pig as consisting essentially in the presence of an abnormal, incompletely developed male gonad in which generative cells are absent, and in which the interstitial cells show an unusually pronounced development. This maleness of the gonad and especially the marked development of an interstitial gland is associated with deficiencies in the formation of the efferent genital ducts and with a female mammary gland. We interpreted this unusual condition as due to the original female constitution in the animal, to the tendencies toward femaleness which prevented the male interstitial cells from exerting their specific influences on the mammary gland, If this guinea pig should represent an analogue to the freemartin in cattle, we would have to assume that the male characteristics of the gonad were produced in an original female through the influence of the testicular substance of the male brother. While such an origin has been made possible through the findings of Lillie, the essential point is that, whatever the origin of this condition, we have every reason to consider the gonad which we described as male.

It is the complete examination of the gonads in serial sections which makes it possible for us to exclude the presence of ovarian structures in this animal and to prove the similarity of the organs with the testicles found under certain experimental and natural pathological conditions. There is no reason for assuming that the gonads in our case represented really ovarian tissue. We have examined many hundred of ovaries of guinea pigs of various ages and have never been able to detect in the ovaries of guinea pigs structures resembling the interstitial gland of the testicle, while on the other hand such structures are strongly developed in testicles which have been retained in the abdominal cavity.

The findings of Lillie and Chapin suggest very strongly, and I take this also to be the interpretation of Lillie, that even in the freemartin the gonad which originally was destined to become female, assumes at least in part true male characters. If this were not so, it would be difficult to explain why seminal vesicles could develop in a freemartin. We should expect that the autogenous ovarian substances would prevent the heterogenous testicular substances from exerting such an influence on the secondary sexual organs of the female twin.

We found in our case a marked development of sexual instinct associated with the absence of generative cells and with a very marked development of the interstitial tissue. Now, as we have pointed out in previous papers, it is improbable that in the guinea pig the interstitial gland is responsible for the manifestation of sexual desire in the female.[1]

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[1] Loeb, Leo, *Zentralblatt f. Physiologie*, 1911, XXV., No. 9, and Loeb, Leo, "The Relation of the Ovary to the Uterus and Mammary Gland from the Experimental Aspect," *Surgery, Gynecology and Obstetrics*, 1917, XXV., p. 300. (A review of the literature concerning this and other aspects of the mechanism of the sexual cycle.) In accordance with the most widely accepted view we assumed in our

Typical interstitial cells are absent in the normal ovary of the guinea pig. If we should assume the gonads in this guinea pig to be female our own observation would constitute a decisive proof that also in the female guinea pig the interstitial cells produce the substance which is responsible for the sexual desire—a conclusion which, however, as we stated above, would be contradictory to other facts which we established previously.

The case which we described may be compared to a single equation communicated by nature, in which two variables are present. While one equation is not sufficient to determine the unknown factors in such a case, the variables have at least been carefully defined in our case. We may hope that later a second equation will be found which will permit defining all the variables. We thought it therefore of interest that these observations should be published.

discussion that it is the so-called interstitial gland of the testicle which is responsible for the development of the secondary sexual characters and of sexual desire in the male. We wish, however, to emphasize the fact that in our case epithelial elements were present in the testicle in addition to the interstitial gland and apriori we cannot exclude the possibility that they and not the interstitial gland are the causative agency in determining sexual phenomena. We are likewise not certain that this can be excluded in those experimental and pathological conditions in which the changes were attributed to the interstitial gland. However we are not directly concerned with this question and we are not in a position to contribute definite data which would favor or contradict this view. We wish, however, to reiterate the fact that on various occasions we made observations which do not seem to accord with the assumption that in the ovary the so-called interstitial gland initiates heat.

PHYSIOLOGICAL SENESCENCE IN HYDROMEDUSÆ.

C. M. CHILD.

The following observations on senescence in the hydromedusæ were made at the Puget Sound Marine Station, Friday Harbor, Wash., during the summer of 1917. I wish to acknowledge my obligation to the director of the laboratory, Dr. Frye, for the use of a room and the privileges of the station, and for his kindness in providing special glassware and other equipment. To other members of the staff I am also deeply indebted for many courtesies.

The great abundance, variety and large size of hydromedusæ at Friday Harbor makes this an extremely favorable locality for work on these forms. During the summer of 1917 five species of hydromedusæ, *Phialidium gregarium*, *Æquorea cærulescens*, *Sarsia rosaria*, *Mitrocoma discoidea* and *Stomatoca atra* could readily be obtained in considerable numbers, often by the hundred, from the laboratory float, and five other species of other genera were more or less abundant, besides two species of siphonophores. The first four species named above furnished the material for the observations and experiments on ageing, and the results are essentially the same in all.

The work was done entirely on free-swimming medusæ and is essentially a comparison of younger and older individuals, no attempt having been made to obtain the medusa-bud stages before detachment from the hydroid.[1] In the selection of individuals of different age for experiment, size of the animal and degree of development of the gonads were used as superficial criteria of age. As might be expected, these two criteria are in general in close agreement, for since the range in variation in size of the medusæ-bud at a given stage of development is not very great, a larger medusa must in general have undergone a greater amount of growth than a smaller, and may therefore be expected, if age differences exist, to be physiologically older. As has been shown for *Planaria*, and other simple animals (Child, '15, Chapter IV.—VII.) physiological age is not necessarily a function of the length of time which the individual has lived, for nutrition, agamic reproduction and various other factors play a rôle in determining the physiological age at any given time. Size is therefore a far more adequate criterion of age in such forms than time. Moreover, since the development of the gonads in medusæ progresses from a relatively early stage to sexual maturity the condition of the gonads also serves as a measure of the stage in its life history which the animal has reached.

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[1] In *Pennanria* a progressive change in physiological condition has been observed from earlier to later stages of medusa-buds (Child, '15, pp. 150–152).

The chief method employed was that of determining the susceptibility of younger and older animals to various agents. The general relation between susceptibility and age has been discussed elsewhere (Child, '14, '15) and need only be briefly restated. In general, in concentrations or intensities sufficiently high to kill without permitting acclimation, the susceptibility decreases with advancing age. In low concentrations, which permit some degree of acclimation, the susceptibility increases with advancing age because the young individual possesses a greater capacity for acclimation then the older, therefore becomes more rapidly and more completely acclimated and so dies later than the older, or lives indefinitely, while the older dies sooner or later.

It has been shown that a relation exists between susceptibility and metabolic activity, more particularly the oxidative or energy-freeing reactions. In general, susceptibility to the higher concentrations or intensities varies directly, and susceptibility to the lower concentrations varies inversely as the rate of these reactions, because acclimation occurs more rapidly and more completely where metabolic activity is greater. This relation between susceptibility and metabolic condition has been shown to hold good not only for different individuals, *e. g.*, young and old (Child, '15) but for different regions of the body of a single individual (Child, '16*a*).

It does not follow, however, from the existence of a relation between susceptibility and rate of metabolism or of certain metabolic reactions, that all agents for which such a relation exists act directly upon the chemical reactions or that they all act in the same way. Much remains to be learned concerning the nature of the action of external agents on living protoplasm, but the fact is already clear that protoplasmic structure, aggregate condition, permeability, surface tension, etc., are associated with metabolic condition. Susceptibility to a given agent serves at best merely as an indicator of protoplasmic and metabolic condition and tells us nothing concerning the way in which the agent acts. As our knowledge of susceptibility to different agents and conditions increases, we find that under certain conditions different agents give us different results, and such differences in susceptibility relations often serves to throw additional light on the physiological condition of the protoplasm or the action of the agent, or both. The general relations stated above hold for a wide range of agents and conditions, and it is primarily with these general relations that we are concerned in the present paper.

Age-differences in Behavior.

A very conspicuous difference between young and old individuals is the much higher rate of pulsation in the young animal and its progressive decrease with advancing age. Table I. gives characteristic data on this point.

Table I.

Species.	Diameter of Body in Mm.	Pulsations in 20 Ser. at 14° C.
Æquorea cœrulescens	7	40
	20	32
	50	20
Mitrocoma discoidea	18	28
	60	16
Sarsia rosaria	5	40
	10	30

In every species observed, ten in all, a similar difference in the rate of pulsation exists, the rate in the sexually mature animal being in most cases about half that of the animal at the beginning or in very early stages of gonad development. By repeated stimulation the rate may often be somewhat increased, particularly in old animals, but the differences in animals under ordinary conditions are of the order of magnitude indicated. This difference in pulsation rate in itself indicates a very marked decrease in metabolic rate with advancing age. Mayer ('06) has noted that in the scyphomedusa *Cassiopeae* the rate of pulsation decreased with advancing age.

Young and old animals also differ in their behavior in another way. Under the usual conditions periods consisting of a larger or smaller number of pulsations alternate with periods of rest. The total length of pulsation periods as compared with rest periods decreases with advancing age; in other words the younger animal spends a larger portion of the time than the older in rhythmic pulsation. Pulsation periods may either be longer or more frequent or both in the younger animal than in the older. In *Æquorea* this difference is perhaps even more strongly marked than in other forms observed. In the large, sexually mature animals 60–80 mm. in diameter, when under natural and so far as possible constant conditions, the periods of quiescence are often one or two minutes long, sometimes even longer, the animal drifting passively during this time without a single pulsation. Following such a quiescent period a single pulsation or a pulsation period may occur. Under the same conditions the quiescent periods in young animals 15–20 mm. in diameter are usually very much shorter, commonly only a few seconds and the pulsation periods are usually longer than in the old animal. The young animal is then more continuously active and gives the impression of a much greater degree of "spontaneity," since it is usually impossible to distinguish any external exciting factor responsible for the beginning of pulsation after a period of quiescence. In the other species observed similar differences between young and old exist, but periods of quiescence are usually shorter than in *Æquorea*.

The irritability of the young animal as indicated by the effectiveness of direct mechanical stimulation in inducing pulsation during a quiescent period is distinctly greater than that of the old. Usually the quiescent animal in the earliest stages of gonad-development responds by pulsation to the slightest touch in the marginal region, while the sexually mature animals commonly respond only to much more intense mechanical action.

These differences between younger and older medusæ show of course a considerable range of variation in different individuals, but are nevertheless striking features of the behavior of these animals when attention is directed to them and they remind one irresistibly of age differences in behavior in much higher animals. The apparent restlessness and spontaneity, the more rapid pulsation rate, and the greater irritability of the young animal as compared with the old all suggest that in these, as in other organisms, so for as known, the life history of the individual is from an early stage a process of physiological senescence which expresses itself dynamically as a decrease in rate or intensity of the fundamental energy-liberating metabolic reactions.

Susceptibility.

In determining the susceptibility of the animals various other criteria besides death and disintegration may be used as a check, viz., cessation of rhythmic pulsation, the disappearance of muscular contractility and shrinkage of jelly in some agents. As regards the relation between susceptibility and age all of these criteria give the same results. It is usually difficult or impossible to determine the exact time when an animal ceases to respond to stimulation by rhythmic pulsation or by slow muscular contractions and the early stages of shrinkage and of disintegration are not less difficult to determine. The times given in the following tables represent approximations only. Frequently pulsation or contractility is present at one observation and absent at the next, and the time of its disappearance can only be estimated. Nevertheless there is no difficulty in distinguishing the differences in susceptibility between young and old animals. The experiments were mostly performed with single pairs, one young, one old, but in some cases two or three of each were used.

Æquorea cærulescens.

This, being one of the most abundant species, was used to a large extent. The smallest and youngest individuals found were 8–10 mm. in diameter, without gonads, the largest, and oldest, sexually mature animals 60–75 mm. in diameter. The chief results are tabulated below. In these tables the sign ± following a given time indicates that this is estimated from two observations between which the change in question occurred, and . . . ? indicates that the change or process did not begin until after the close of the experiment. In this species cessation of pulsation, disintegration and in some cases loss of contractility are available as criteria of susceptibility. The jelly is very firm and does not shrink to any great degree in any agent used.

The three tables agree in showing a greater susceptibility in young than in old animals, as indicated by cessation of rhythmic pulsation, loss of muscular contractility and disintegration. Special attention must be called to certain points. In Table II. it is of interest to note that KNC *m*/10000 is about as effective, or in some cases more effective than much higher concentrations, *m*/500, *m*/200, in stopping pulsation. It has been observed in other cases (Child, '16*a*, '16*b*) that rapidity of action of KNC and certain other agents, as indicated by death or other effects, increases only slightly with increase in concentration above a

TABLE II.

Æquorea: SUSCEPTIBILITY TO KNC.

Series.			Diameter in Mm.	Conc. KNC.	Duration of Experiment.	No Pulsation after Stimulation.	No Contraction after Stimulation.	Disintegration.
1		*A* . .	7	*m*/10000 After neutral red	1 hr.	1 min. ±	5 min. ±	. . . ?
		B . .	12			5 “	10 “ ±	. . . ?
		C . .	20			10 “	20 “	. . . ?
		D . .	50			40 “	60 “ ±	. . . ?
2		*A* . .	12	*m*/10000	4 hrs.	30 sec.		. . . ?
		B . .	30			20 min.		. . . ?
7	I.	*A* . .	18	*m*/500	17 hrs.	2–5 min.		12 hrs. ±
		B . .	60			10–15 “		17 “ ±
	II.	*A* . .	18	*m*/200	6 hrs.	3 min.		4–6 hrs.
		B . .	60			8 “		6–? “
	III.	*A* . .	16	*m*/100	2 hrs.	At once		1 hr.
		B . .	70			“ “		2 “
	IV.	*A* .	20	*m*/100	3 hrs.	At once		$1\frac{1}{4}$–$1\frac{3}{4}$ hrs.
		B . .	60			“ “		$1\frac{1}{2}$–3 “

certain limit or may even decrease. The facts suggest that the high concentrations may decrease permeability to themselves, at least up to a certain limit where their action becomes violently destructive to protoplasm, and so practically instantaneous.

In Table III., HCl and Table IV. KOH, the action in stopping pulsation increases very rapidly with increasing concentration, proportionately much more rapidly than the concentration, because more or less acclimation to the lower concentration occurs. In HCl *m*/1500—not tabulated—acclimation occurs in the young animal to a greater degree than in the old. After five days in this concentration the young animal still shows well-marked rhythmic pulsation after mechanical excitation while

Table III.

Æquorea: Susceptibility to HCl.

Series.		Diameter in Mm.	Conc. HCl.	Duration of Experiment.	No Pulsation after Stimulation.	No Contraction after Stimulation.	Disintegration.
II.	*A*..	18	*m*/1000	5 days	4½ days	...?	...?
	B..	60			...?	...?	...?
III.	*A*..	15	*m*/1000	5 days	3 days −	4 days +	...?
	B..	40			...?	...?	...?
3 IV.	*A*..	18	*m*/800	5 days	3 days +	4 days ±	...?
	B..	60			...?	...?	...?
V.	*A*..	18	*m*/500	21 hrs.	At once		8–12 hrs.
	B..	60			1 min. ±		17 hrs. ...?
4	*A*..	18	*m*/500	1 hr.	At once		
	B..	60			2 min.		
6	*A*..	18	*m*/400	1 hr.	At once		55 min. ..?
	B..	60			" "		...?
I.	*A*..	18	*m*/300	2¼ hrs.	At once		55 min. ..?
	B..	60			" "		2¼ hrs. ..?
5 II.	*A*..	18	*m*/300	2¼ hrs.	At once		
	B..	60			1 min.		

the old animal cannot be induced to pulsate. In concentrations of *m*/1000 or higher, the young animal is always the more susceptible (see Table III.).

The records on the loss of muscular contractility are very incomplete, but it is evident that rhythmic pulsation and general muscular contraction are not the same thing. Contractility of the muscles of the umbrella persists in all three agents long after ability to pulsate has disappeared, except in concentrations so high that death is practically instantaneous.

TABLE IV.

Æquorea: SUSCEPTIBILITY TO KOH.

Series.		Diameter in Mm.	Conc. KOH.	Duration of Experiment.	No Pulsation after Stimulation.	Disintegration.
9 IV.	*A*. .	16	*m*/1000	84 hrs.	12 hrs. ±	48 hrs. ±
	B. .	60			48 hrs. ±	. . . ?
8 I.	*A*. .	18	*m*/800	34 hrs.	10 min.	4–6 hrs.
	B. .	60			24 hrs.	. . . ?
8 IV.	*A*. .	15	*m*/800	28½ hrs.	5 min.	3–7 hrs.
	B. .	60			26 hrs. ±	28½ hrs. . . . ?
9 III.	*A*. .	14	*m*/600	9 hrs.	2 min.	4–6 hrs.
	B. .	60			4–5 min.	9 hrs. . . . ?
9 II.	*A*. .	18	*m*/400	9 hrs.	30 sec.	2–4 hrs.
	B. .	75			1 min. +	3–5 hrs. +
9 I.	*A*. .	18	*m*/300	5 hrs.	5 sec. ±	1–2 hrs.
	B. .	70			20 sec. ±	3–5 hrs.
8 II.	*A*. .	14	*m*/200	5 hrs.	At once	2–4 hrs.
	B. .	60			10–20 sec.	3–5 hrs.
8 III.	*A*. .	16	*m*/200	3½ hrs.	At once	1¼–2¼ hrs.
	B. .	60			20–30 sec.	2–3½ hrs.

A complete but temporary inhibition of pulsation lasting from a few seconds to several hours occurs in almost all cases in HCl *m*/600. Above *m*/600 inhibition is usually permanent. At the lower limit of concentration for temporary inhibition only the young member of a pair is inhibited; with somewhat higher concentration both are inhibited for a short time but the young animal usually resumes pulsation before the old if the differences in age are not too extreme. As the concentration increases, the length of the inhibition period increases more rapidly in the young than in the old, until about the upper limit permanent inhibition occurs at once in the young animal while some slight pulsation may reappear sooner or later in the old. In short the age differences in susceptibility appear here as in other features of the action of external agents.

Experiments on recovery after temporary exposure to an agent showed that the relation between recovery and age is the same as in other forms examined. The reappearance of the pulsation response to stimulation is the criterion of recovery used. Where the concentration is not too high, the exposure too long or the difference in age between the animals too great, the young will recover before the old on return to water, but with higher concentrations longer exposures and greater differences in age the old animal recovers earlier and usually more completely than the young. These relations between recovery, age, concentration and period of exposure are of course merely a special case of the age-susceptibility relation. To higher concentrations or longer times of exposure the young animal is more susceptible than the old, but to low concentrations or short times of exposure it is able to adjust itself more rapidly or to recover more rapidly afterward than the old (Child '15, '16*a*). The degree of difference between young and old animals also differs widely with different agents, because acclimation to some agents occurs much more rapidly than to others. The observations on recovery and its limits are as yet only fragmentary and further work is necessary before a complete statement can be made.

Some of the data of an experiment with HCl *m*/500 will serve as an example of recovery. Animals 15 mm. and 60 mm. are placed in HCl *m*/500 and at intervals a pair, one of each size, is returned to water. In the young animals all pulsation ceases at once in HCl, in the old it ceases after two minutes. On return to water after 5–10 minutes in HCl pulsation usually reappears after 2–3 minutes in both old and young, often slightly earlier in the young. After one hour in HCl pulsation reappears after 10 minutes in the old, and after 12–13 minutes in the young and after 2½ hours in HCl pulsation reappears in the old after 25, in the young after 45 minutes. The longer the exposure the more the young animal falls behind the old in recovery. After 20 minutes in HCl *m*/400 young animals 18–20 mm. did not recover at all while old animals 60 mm. showed slight pulsation after four hours in water. Recovery after KNC is much slower and occurs only in much lower concentrations.

In all concentrations of KOH from *m*/1000 to about *m*/250 a primary acceleration of pulsation rate and increase in strength of contraction occurs, but in concentrations higher than *m*/250 pulsation usually ceases at once or in a few seconds. During this primary acceleration the pulsation rate is often doubled, but since the maximum rate usually persists only a few seconds and is followed by progressive decrease in rate it is difficult to determine whether the degree of primary acceleration differs in a characteristic way with age. It is certain, however, that the retardation following the acceleration occurs more rapidly in young than in old animals, so that pulsation ceases first in the young, as Table IV. indicates.

The opposite primary effects of acid and alkali on the pulsation rate are what might be expected in the light of what we know of their action on living protoplasm in general. In lethal concentrations, however, their action, following the primary effect, is essentially similar and the relations between susceptibility and age are the same for both.

Mitrocoma discoidea.

In this species the jelly is much less firm than in *Æquorea*, and marked shrinkage occurs in the young animals in acids and some other agents. Muscular contraction as distinguished from pulsation is much more frequent even in nature than in *Æquorea*. Frequently contraction brings about a folding of the umbrella with the two halves of the margin approximated or apposed along a straight line, or the margin may become square or very irregular in outline. The contracted condition may persist for several minutes, or after very strong stimulation an hour or two before return to the usual form and resumption of pulsation occur. As in *Æquorea*, the ability to contract persists long after the ability to pulsate has disappeared.

In Table V. the chief data obtained with HCl are given. They are essentially similar to those for *Æquorea*.

TABLE V.

Mitrocoma: SUSCEPTIBILITY TO HCl.

Series.		Diameter in Mm.	Conc. HCl.	Duration of Experiment.	No Pulsation after Stimulation.	No Contraction after Stimulation.	Beginning of Shrinkage.	Disintegration.
X.	*A*	18	*m*/1500	114 hrs.	. . . ?	Acclimated: active at end of expt.		
	B	60			114 hrs. −	. . . ?	. . . ?	. . . ?
II.	*A*	18	*m*/1000	55½ hrs.	40 hrs. ±	. . . ?	. . . ?	. . . ?
	B	60			. . . ?	. . . ?	. . . ?	. . . ?
III.	*A*	18	*m*/800	48 hrs.	8½ hrs.	24 hrs. +	24 hrs.	40 hrs. . . . ?
	B	55			40 hrs. ±	. . . ?	. . . ?	. . . ?
IV.	*A*	15	*m*/700	48 hrs.	At once	24 hrs. ±	30 hrs. ±	40 hrs. . . . ?
	B	60			15 hrs.	40 hrs. ±	. . . ?	. . . ?
5 V.	*A*	20	*m*/650	34 hrs.	2 hrs.			30 hrs. . . . ?
	B	45			15 hrs. ±			34 hrs. . . . ?
VI.	*A*	18	*m*/650	52½ hrs.	26 hrs. ±	24 hrs. ±	22 hrs.	45–52½ hrs.
	B	60			34 hrs. ±	40 hrs. ±	. . . ?	50 hrs. . . . ?
VII.	*A*	18	*m*/600	51½ hrs.	At once	24 hrs. ±	32 hrs.	45–51½ hrs.
	B	60			26 hrs. ±	. . . ?	. . . ?	. . . ?
VIII.	*A*	18	*m*/500	4¼ hrs.	At once	1 hr. ±	2 hrs.	
	B	60			" "	. . . ?	. . . ?	
IX.	*A*	20	*m*/500	9¾ hrs.	" "	At once		
	B	60			" "	. . . ?		

The first series 5, X. in HCl *m*/1500 shows acclimation as regards the effect on pulsation rate, pulsation persisting longer in the young than in the old. In *Æquorea* also some degree of acclimation usually occurs in this concentration (p. 53). The long persistence of pulsation in both members of the pair in Series 5, VI. (HCl $m/650$) as compared with the other series is anomalous and no reason for it is known. Otherwise the data in the table are consistent and show that for the concentrations included, except $m/1500$, the young animal is more susceptible than the old.

Results with KNC show similar relations between age and susceptibility, of course with much lower concentrations. The results of one series on pulsation susceptibility are given in Table VI.

TABLE VI.

Mitrocoma: SUSCEPTIBILITY OF PULSATION TO KNC.

Series.		Diameter in Mm.	Conc. KNC.	Duration of Experiment.	Pulsation.
4. IV.	*A*..	18	$m/100000$	5 hrs.	Pulsation only retarded: rate $A = \frac{1}{2}$ rate B.
	B..	60			
4. III.	*A*..	18	$m/50000$	$5\frac{1}{2}$ hrs.	Very slight, irregular.
	B..	60			Well-marked, regular.
4. I.	*A*..	16	$m/25000$	$\frac{1}{2}$ hr.	Ceases in 10–15 min.
	B..	60			" " 15–20 "
4. V.	*A*..	18	$m/10000$	$\frac{1}{2}$ hr.	Ceases in 5 min. ±
	B..	55			" " 15 " ±

In the first pair, 4, IV., $m/100000$, the susceptibility is shown, not in the time of cessation of pulsation, but in the retardation of the rate. At the beginning of the experiment rate $A = 2$, rate B approximately, but after five hours rate $A = \frac{1}{2}$ rate B. In the second pair, 4, III., $m/50000$, pulsation in the young animal is almost completely inhibited after $5\frac{1}{2}$ hours, while in the old it is still strong. Here rate A was very much lower than rate B. In the higher concentrations pulsation ceases in a few minutes.

A few experiments with other agents give similar results. In neutral red animals 18–20 mm. long cease to pulsate, undergo shrinkage and begin to disintegrate much earlier than animals 60 mm., although no marked difference in rate or intensity of staining is apparent. In methylene blue 1/30000, increased to

1/15000 after 24 hours, the animals live for several days with all cellular tissues stained. After 48 hours pulsation rate $A = \frac{1}{2}$ pulsation rate B approximately, where A and B are animals of 18–20 and 50–60 mm. respectively.

Phialidium gregarium.

The youngest animals found were mostly 7–8 mm. in diameter, while the old, sexually mature individuals measured 20–25 mm. Only a few series of susceptibility experiments were made with this species, HCl *m*/1500, *m*/1000, *m*/800, *m*/600, neutral red and methylene blue being used. The susceptibility relations are similar to those observed in the other species. In this rather delicate form shrinkage is a characteristic feature of the action of various agents, the younger animals undergoing shrinkage earlier and to a greater degree than the old, a decrease of half or three fourths in diameter occurring in many cases.

Sarsia rosaria.

In this species the umbrella is very deep, the oral-aboral axis being almost or quite twice the diameter. Depth is therefore a more convenient measure of size than diameter and is commonly used. The sexually mature animals average about 15 mm. in depth of umbrella, but the extremes range from 10–12 mm. on the one hand to 20 mm. on the other. In the smallest and youngest animals found, 6–7 mm. in depth, the gonads had already begun to develop, and although the susceptibility of these forms is characteristically greater than that of the mature individuals the differences in age and in susceptibility in the animals available are not as great as in the other species examined.

In general, results with this species are similar to those with others but in some pairs an individual 12–15 mm. showed a susceptibility equal to or even greater than that of 7–8 mm. animals. Since none of the animals within these limits of size represent very great differences in physiological age, all being near sexual maturity, it is to be expected that occasionally a larger animal will show a higher susceptibility than a smaller.

The agents used were KNC *m*/50000, *m*/10000, *m*/5000, HCl *m*/1000, *m*/600, *m*/500, *m*/400, neutral red and methylene blue, and the age differences appeared in all with the few exceptions above noted. One of these exceptions was rather remarkable. In KNC *m*/50000 animals 7–8 mm. usually lose the ability to pulsate, even on stimulation, in 3–10 minutes, animals 15 mm. after 1–2 hours, while in *m*/10000 the times for cessation of pulsation are respectively 1–2 minutes and 3–10 minutes. In one case, however, in KNC *m*/10000 an animal 15 mm. ceased to pulsate in three minutes, while an animal 8 mm. in the same closed container continued normal pulsation during thirty minutes, longer observation being impossible. For some reason this particular animal was practically insusceptible to KNC *m*/10000, a concentration which stops pulsation in the younger individuals of the other species examined in 5 minutes or less and in other younger individuals of *Sarsia* in 1–3 minutes. This extreme exception is merely recorded without any attempt to account for it. The observations on *Sarsia* do indicate a wider range of physiological condition in animals of a given size than in the other species examined, but they afford no clue to interpretation of a condition so extremely exceptional as this.

Conclusion.

The preceding data make it evident that the hydromedusa, like other animals, undergoes a progressive change in physiological condition with advancing development, the differences in behavior and susceptibility being indicators of this change. It has been shown that in other forms a decrease in the rate of oxidations is a characteristic feature of the change and the facts indicate that a similar decrease occurs here, although these forms are not very favorable material for the direct determination of oxygen consumption or CO_2-production. The assumption that the differences in susceptibility are due merely to differences in permeability of surface membranes is refuted by the results obtained with neutral red and methylene blue and by the cases of acclimation. Moreover, there is every reason to believe that the age differences in susceptibility to cyanides are at least more directly associated with the oxidation rate than with permeability.

As regards permeability, however, it is becoming more and more evident to investigators in this field that the condition of the membrane is not independent of the metabolic condition of the protoplasm. Permeability to various agents is itself within certain limits an indicator of age or physiological condition of the cell.

The progressive change in behavior, the decrease in pulsation-rate and "spontaneity" indicate very clearly a process of physiological senescence and the data on susceptibility only confirm and extend the facts of observation.

SUMMARY.

Four species of hydromedusæ show evidence of a process of physiological sensecence in the decrease in pulsation-rate and apparent spontaneity and in the decrease in susceptibility to KNC, HCl, KOH and "vital" dyes in various concentrations with advancing development. The criteria of susceptibility are change in rate or cessation of pulsation, loss of contractility, shrinkage of the jelly and disintegration of cellular tissues, and all these criteria agree. The facts justify the conclusion that a decrease in the rate of metabolism, or more specifically of oxidations, is a characteristic feature of this process of senescence.

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BIOLOGICAL BULLETIN

HEREDITY FROM THE PHYSICO-CHEMICAL POINT OF VIEW.[1]

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Heredity, the power of reproducing its like, is a property of all forms of living matter from the lowest to the highest. Broadly speaking, whenever a universal property or mode of action is found, the presumption is that its basis is fundamentally *simple*, for repetition is characteristic of simple rather than of complex conditions and objects in nature: in general, the fewer the variables the more constant the phenomenon. We have therefore to seek for some general or fundamental structural or physico-chemical peculiarity of living things which enables their substance to build up substance of a similar kind. Any form of protoplasm acts as a center of construction of similar forms. At present we are not concerned with the further fact that this reduplication is not perfect, that varieties appear and that diversity has arisen in the course of evolution. This process of divergence is gradual, and even in mutants the differences from their parents are slight compared with the resemblances; the essential fact is that the type is preserved, and that normally the organism appears unable to construct living matter of other than its own kind. The main question is why any species of organism tends so strongly to retain its specific character.

Apparently the most universal property of living matter is its power of proliferation. Out of materials and energy taken from the surroundings it constructs more living matter of the same kind. In other words, the power of *growth* is innate; and if the available nutritive and other materials are sufficient the quantity of the specifically organized and active living substance tends continually to increase. The degree of increase possible under the conditions may be limited; and in fact most individual organisms and cells have definite limits of size, a balance being eventually reached where metabolic destruction balances construction; nevertheless the construction of new living substance, the essential process underlying growth, continues in all cases throughout life, and when it ceases, life ceases; the constructive process cannot suffer permanent interruption. Even where the organism as a whole seems to have reached its final dimensions there are always special cells or regions which when placed under appropriate conditions still show indefinite proliferative activity. In higher organisms such regions are represented more especially by the germinal epithelium; and a single detached cell from this region exhibits under certain conditions that unique property of definite and orderly proliferation known as development, which leads to the production of an organism similar in its minutest details to that of which the cell originally formed part. This is the chief form which the process of reproduction takes in higher animals, and such a manner of consideration shows that no fundamental distinction can be drawn between growth and reproduction. In many organisms almost any portion of sufficient size which is detached from the whole—whether by some physiologically normal mechanism like fission or as the result of operation—may continue its growth after isolation, redifferentiate, and eventually regain the form and physiological characters of the original stock. Whole plants may thus be reproduced from artificial cuttings, and the same is true of many lower animals (protozoa, hydroids, planarians). In such cases the distinction between growth and reproduction becomes ill-defined, and reproduction appears as essentially a form of discontinuous growth; and in an organism like yeast, where the growing cell-masses may either cohere in chains or fall apart into separate cells—apparently as the result of purely casual conditions—the distinction ceases to have more than a formal significance. If the cell-chain is regarded as the unit organism, increase in its length by budding is a matter of *growth;* if the single cell is so regarded, the same phenomenon becomes an instance of *reproduction.*

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[1] From the Laboratory of General Physiology, Clark University.

It is important for the purpose of the present discussion that the artificiality of this distinction should be recognized at the beginning, in order that the nature of the essential problem should be clearly defined. Our aim is to analyze into its simplest physico-chemical terms—so far as this is possible at present—this power of specific construction, of structural and chemical synthesis, which is possessed by all forms of living matter. Reproduction and growth are different manifestations of the same proliferative process. Gametic reproduction is, to be sure, the most highly involved and specialized form of proliferation which we know; yet even here, as also in the case of a simple yeast-cell or bacterium growing in a culture-medium, the process of construction is accomplished through the action of the original germ in incorporating and transforming physically and chemically, in a definite and specific manner, certain materials (food, water, salts, oxygen) taken from the surroundings. The problem of just why the complex and highly organized living system thus built up from a particular species of egg-cell should exhibit its own specific structural, physiological, and chemical peculiarities, and why these should be identical with those of the parent, is one which can be solved in detail only by the special investigation of that particular species. But the fact that any such special development is a prolonged and complex process, involving a progressively increasing differentiation and at length reduplicating the parent form, does not alter its general character as proliferation. The fundamental *general* problem remains the same, whether the process under consideration is the formation of new yeast-cells or bacteria in a culture medium, or the development of a higher animal from a fertilized egg. In both cases material from the surroundings is transformed into living specifically organized substance of a constitution identical with that of the parent organism. And we have to ask whether it is possible, in the present state of our knowledge, to form any clear and consistent conception of the general nature of the physico-chemical conditions under which such a result is accomplished.

A simple concrete instance will define more clearly the nature of the problem to be solved. Consider the case of a single yeast cell introduced into a culture medium. The cell grows and forms buds; these give rise to other buds, and cell-chains are formed from which single cells detach themselves and pass through similar transformations; eventually in place of the original cell there are thousands. The non-living material of the culture medium—consisting of water, sugar, ammonium tartrate, and inorganic salts of potassium, calcium, iron, and magnesium—has been transformed into complex and active living protoplasm of a specific chemical and structural organization. Each living cell exhibits the "germ-action" so characteristic of life—*i. e.*, acts as a center of chemical and physical transformation of a definite kind by which more and more yeast protoplasm is formed. We may note here the analogy—to which we shall return—with the process of specific accretion by which a crystal introduced into a super-saturated solution increases its size and becomes a center of deposition of more crystals of the same kind. But the analogy is incomplete; the living organism does not merely change the physical state of the substances which it takes in from the surrounding solution; it also modifies them chemically in the most profound manner, and from the above simple materials it builds up proteins, lipoids, fats, glycogen, and a multiplicity of other substances not present in the culture medium. Further, not only are these substances synthesized, but they are distributed throughout the growing mass of protoplasm in a perfectly definite manner, partly as solid structural material, partly as dissolved or other material serving for energy-production or some form of metabolism.[1] Each one of the specific organized structures thus produced, the yeast cells, reduplicates the physiological activities as well as the structural characters of the parent cell. The result is that the non-living material of the surroundings is transformed or worked over into organized living material of a predetermined type, *i. e.*, the transformation is *specific* and depends upon the nature of the germ originally introduced into the medium. Both growth and heredity are exemplified in their simplest manifestations; and we see again that these terms do not signify two objectively different processes, but merely two aspects of the same process—"growth," the quantitative term, denoting the increase in the total mass of living substance, while "heredity" emphasizes the specificity of the process and its dependence upon the parental character.

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[1] *I. e.*, the problem of *differentiation* is inseparable from the problem of growth.

It is possible to say that each yeast cell *inherits* its properties from the mother cell; but such a phrase serves merely to indicate that the character of the transformation depends upon the character of the germ or proliferating organism itself, rather than upon the character of the material which is transformed under its influence. A mould or bacterium in the same medium effects a totally different kind of transformation, but one equally specific and equally true to the character of the introduced germ.

In general the possession of this automatic property of specific structural and chemical synthesis constitutes the most fundamental distinction between the living and the non-living material systems found in nature. We observe that all organisms and all living cells without exception possess this power; they transform certain inert materials selected from the surroundings into their own characteristically organized and physiologically active living substance. The materials thus incorporated and transformed differ widely in their character and accessibility in different organisms; at the one extreme are green plants living on carbon dioxide, salts, and water; at the other extreme is man in his complex social environment. But this difference of degree does not alter the fundamental identity of kind. Wherever we find life we find exhibited this unique property of specific construction or synthesis, whether the product of the construction is simple or complex.

This property also manifests itself in another and less evident manner. The specific organization of any animal or plant, with the associated physiological activity, not only *originates* in this manner as the product of growth from the parent organism, but once reached, it has to be *maintained.* And this maintenance involves the activity of specific construction in just as full a sense as does growth or development from the germ. During life the organized living substance is continually being destroyed, and must as continually be replaced. This becomes especially evident in certain of the lower animals whenever the food-supply is withdrawn; the quantity of living substance then undergoes progressive reduction, which may proceed until only a small fraction of its original quantity is left; a planarian may thus be reduced by starvation to a quarter of its original length without injury, but disorganization and death follow further reduction unless food is restored. A similar loss of living substance through the destructive metabolism inseparable from the life-process takes place in all cells, although its rate and its possible limits vary in the different cases—apparently because of the unequal resistance of different structural elements to regression of this kind; thus in higher animals the loss during starvation is great in voluntary muscle and small in the heart and nervous system. In all cases the specific structural material appears subject to a certain continual breakdown of this kind; even when the non-nitrogenous food-supply is ample for energy-requirements it is found impossible to reduce the nitrogen metabolism—the index of destruction of protein, the specific structure-forming substance —below a certain well-defined minimum. This can only mean that the structural or organized material is subject to continual destruction, and that maintenance involves its continual replacement. This process of replacement is specific, in precisely the same sense in which the growth-process is specific. We must therefore recognize that maintenance involves the activity of specific chemical and structural synthesis in the same sense as does growth. When the construction of organized substance balances destruction there is equilibrium—the condition corresponding to nitrogenous equilibrium in higher animals; any excess of construction leads to growth, of destruction to regression. We see once more that what is essential to continued life is the specific synthetic activity of the protoplasm; when this ceases, life ceases. Claude Bernard expresses this necessary dependence of life upon synthetic or creative processes in the phrase, "life is creation." It is clear that the process of specific creative synthesis which lies at the basis of heredity is inherent in the life-process in all of its forms. The problem of heredity is not a problem to be dealt with by itself; it becomes identical with the most fundamental problem of general physiology, the problem of how living protoplasm is synthesized from non-living matter.[1]

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[1] All of this is clearly recognized by Claude Bernard; *cf.* "Leçons sur les phénomènes de la vie," Vol. 2, p. 517, where he summarizes his general view as follows: "The synthetic action by which the organism thus maintains itself [*i. e.*, by a combination of chemical and formative synthesis] is at bottom of the same nature as that by which it repairs itself after it has undergone mutilation, or still further,

The general physiological problem presented by the phenomena of growth and heredity thus reduces itself to these terms: what are the essential physico-chemical conditions upon which this power of specific construction depends? It seems clear that only a thorough knowledge of the conditions determining the special type of metabolism involved in the process, and especially of its constructive side, can answer such a question. The living organism or cell is primarily a metabolizing system. Growth is the expression or result of a process of metabolism; both the material and the energy required for growth come from outside; within the organism they are transformed in such a manner as to build up an organized system of predetermined kind, the seat of chemical and physical processes which maintain the system and enable it under appropriate conditions to increase in size or to produce other similar systems leading independent life.

In all organisms constructive metabolism involves the synthesis of a multiplicity of new chemical compounds from the food and other materials furnished by the surroundings. The food-materials are typically non-specific in their chemical nature, *i. e.*, they show no relation to the specific character of the organism utilizing them; they are either chemically simple in themselves, or become so during incorporation. In animals, where the organism receives part of its food in highly specific form, as protein, all specificity is invariably lost in the hydrolytic processes of digestion; the material before becoming available for nutrition is reduced to a form in which it can be utilized indifferently by all cells. This non-specificity of the food-materials, as they reach the cells, is in striking contrast with the specificity of the compounds built up from them within the cells. Reduction to a simple or non-specific state is thus the indispensable preliminary to the constructive process. It is therefore highly significant that the chief structural colloids, the proteins, are so readily transformed from the specific to the non-specific state.

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by which it grows and reproduces itself. Organic synthesis, generation, regeneration, maintenance, healing of wounds, are different aspects of an identical phenomenon, are varied manifestations of the same agent. . . ." In the Presidential address of J. S. Haldane before the British Association in 1908 (Nature, Vol. 78, p. 555) a similar point of view is expressed, *e. g.* "nutrition itself is only a constant process of reproduction . . . . Heredity is for biology an axiom and not a problem."

The universal presence of proteases in cells seems to be the expression of this necessary condition, since in all cells the structural proteins are capable (under certain conditions) of regression and translocation, *i. e.*, of being utilized as food-material elsewhere—the proteins of one region of the cell or organism acting as reserve, so to speak, for construction at other regions. Such a condition is apparently necessary for the normal regulation of cell-structure and activities. For the continuance of normal cell-activity a proper ratio between structural and metabolizing substance must be preserved;[1] hence in every cell the conditions must be present for reducing proteins and other materials to a non-specific and diffusible form, in addition to the conditions for specific synthesis.

By the synthetic activity of the protoplasm these relatively simple substances are united and chemically remodeled so as to form a variety of more complex compounds of which the most individualized and specific are the proteins. The term *specific* is here used as meaning peculiar to the particular organism or cell under consideration, and not occurring elsewhere. It is not a coincidence that living organisms, the most complex systems, in the structural sense, occurring in nature, are also the most complex in the purely chemical sense; and all of the evidence indicates that the structural complexity is the expression or consequence of the chemical complexity.[2] The essential reason for this appears to be that a high degree of chemical specificity or individualization is the necessary prerequisite for structural complexity, and that chemical specificity depends largely upon peculiarities of stereochemical configuration. The number of individualized isomers in the case of any organic compound increases rapidly with increase in the number of asymmetric carbon atoms in the molecule. Hence the proteins, formed of linked amino-acids, most of which are asymmetric compounds, exhibit the possibilities of chemical individualization to a greater degree than any other known class of compounds. It is further significant that proteins which are specifically distinct chemically, although otherwise closely similar—*e. g.*, hæmoglobins from different species—tend to form crystals, *i. e.*, structural aggregates, which are specifically distinct in their form-characters.[1] A definite relation between the chemical specificity and the structure-forming properties of these colloids is thus indicated. The relation of proteins to organic structure therefore requires special consideration in any theory of heredity.

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[1] *E. g.*, in a starving protozoön or planarian the normal structure and proportions are preserved, in spite of the decrease in size.

[2] Similarly with structural diversity, whether in the same organism or in different organisms. A corresponding chemical diversity is implied.

The proteins form the colloids out of which, together with certain other associated materials, chiefly lipoid, the more permanent or stable, *i. e.*, "structural" portions of most living organisms, and especially the cell-structures, are built up. The relations of these substances to the specific characters of the organism must for this reason be recognized as peculiarly intimate, even though we are not yet in a position to understand the exact relationship between a particular type of structure in an organism and the specific peculiarities of the protein composing that structure. The available evidence indicates, however, that a definite relationship of this kind does exist. We know for example that the presence of foreign proteins is often incompatible with the preservation of normal structure in cells; the cytolytic action of foreign blood-sera, and the formation of specific cytolysins when cell-proteins are used as antigens, show that so fundamental a character as the semi-permeability of the plasma-membrane in a cell is dependent upon the specific peculiarities of its constituent proteins. And in all cell-structures it is probable that a similar relation exists. Such facts indicate clearly that the specific structure of a cell or organism depends upon the chemical specificity of its structural proteins. Now the term specificity, as applied to the individualized character of an organic species, has its morphological and physiological as well as its chemical significance: *i. e.*, each species has its own special form and structure and its own characteristic modes of activity and behavior, as well as its own distinctive and unique chemical composition. But according to the present conception it is the *chemical* specificity which forms the basis of the other two, and chemical specificity is primarily the property of the proteins. Other biochemical compounds appear to be chemically the same wherever found, but the proteins vary in their specific character from species to species. Moreover, physiologically corresponding or "homologous" proteins are more nearly alike in their chemical and physical characters the more closely related the species are from which they are taken. There is thus a general parallelism between the degree of chemical relationship exhibited by homologous proteins from different organisms, and the degree of biological relationship existing between the species. The indications of this are too various to present in detail in this brief paper, and the evidence has recently been reviewed in an admirable manner by Loeb in his "Organism as a Whole."[1] The specificity which such proteins exhibit when used as antigens, *e. g.*, in the formation of precipitins or specific cytolysins, or in the phenomena of anaphylaxis, shows clearly that the proteins of one species are chemically distinct from the corresponding proteins of even nearly related species, and still more distinct from those of more distant species. Nuttall's well-known work shows that the ability of a given precipitin to react with and precipitate its corresponding protein from another species is a close indication of the degree of blood-relationship between the species under consideration.[2]

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[1] *Cf.* Reichert and Brown: "Differentiation and Specificity of Corresponding Proteins and other Vital Substances in Relation to Biological Classification and Organic Evolution: the Crystallography of Hæmoglobins." Carnegie Institution of Washington, 1909.

Apart from these facts, whose significance in relation to the present problem is now well recognized, there are other evidences of chemical specificity in proteins that offer clearer indications of the nature of the connection between the chemical character of a protein and the character of the structures which it forms in the living cell. The work of Reichert and Brown has shown that hæmoglobin crystals from a given species exhibit form-characters which are definite and specific for the species. This means that when the protein separates from solution in the process of crystallization the molecules, as they unite to form larger crystalline aggregates, by degrees build up structures with definite form-characters—the typical forms of one species exhibiting constant differences from those of other species, even of the same genus. The growing crystal takes on a definite "species-specific" form, in a manner suggesting a close analogy to the growing germ; this definite form is the expression of the specific properties of the protein molecule, and is presumably dependent upon its special stereochemical configuration. It is well known that molecules of similar stereo-structure tend to segregate in the process of crystallization; thus in the crystallization of a racemic tartrate from its solution one group of crystals is formed exclusively—or at least predominantly—of molecules of the dextro-tartrate, the other of the lævo-tartrate, although in respect to solubility and other physico-chemical properties the two compounds are identical. In such an instance it is quite certain that similarity of stereo-structure is the critical factor determining the union of the dextro-molecules to form a definite crystalline aggregate specifically distinct from that formed by the lævo-molecules.[1]

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[1] *Cf.* Chapter 3: "The Chemical Basis of Genus and Species."

[2] Nuttall, "Blood Immunity and Blood Relationships." Cambridge University Press, 1904.

The physiological properties of the two stereo-isomers are correspondingly unlike; fermentability and related properties (such as general assimilability and pharmacological action) have been shown to differ markedly in a large number of pairs of asymmetric compounds, a clear proof that the activity of living protoplasm is largely conditional upon the specific space-relations of the atoms composing the physiologically active molecules. This is particularly true of compounds entering into metabolism: thus we know that enzyme action is determined by stereo-structure. Perhaps the clearest proof that specific constructive metabolism is similarly determined is furnished by the specific character of the metabolic response following the introduction of protein antigens into the organism—*i. e.*, by the specific character of the anti-bodies produced. These new compounds, evidently synthesized by the living cells, exhibit specific chemical properties which are directly determined by the specific properties of the foreign protein introduced. The intimate relationship between the phenomena of immunity and the phenomena of normal assimilation has been pointed out by Ehrlich and others. And since heredity is primarily a matter of assimilation—*i. e.*, construction of the like—it seems clear that the construction of specific protein in the normal processes of growth is the expression of a similar determination of specific constructive processes in the cell by means of the proteins *normally* present. In other words, *the structural proteins already present must determine the production of similar proteins.*

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[1] Thus the introduction of a crystal of the lævo form into a supersaturated solution of racemic tartrate causes the separation of lævo-tartrate alone; similarly dextro-crystals separate out dextro-tartrate (*cf.* Gernez: Comptes rendus, 1866, Vol. 63, p. 843). The recent work of Marc indicates that in general crystallization is preceded by an adsorption; and that crystals, when used as adsorbents, adsorb by preference substances which crystallize in a similar form. *I. e.*, the similarity in the spatial configuration of the molecules is what determines their union to form larger aggregates (*cf.* Marc: Zeitschr. physik. Chem., 1913, Vol. 81, p. 641; also *ibid.*, 1911, Vol. 75, p. 710, and earlier papers there cited).

The above tendency of structurally similar compounds to form aggregates when they separate from solution is probably the essential reason why the proteins native to the cell—already forming part of its structure—undergo increase in quantity, with the result that the cell grows. Nothing less than this is to be inferred from the fact that proteins form the basis of specific structures, and that the preservation of the normal characters of any living cell depends upon the continual formation and reformation of these particular compounds. Those proteins which are already laid down as structure within the living cell are thus to be regarded as acting as centres of deposition of further protein identical in composition and configuration. It seems necessary also to conclude that these same structural proteins directly control or guide the actual synthetic process by which more protein of the same kind is built up, presumably by the dehydrolytic condensation of the amino-acids present in the protoplasm. The precise and specific mode of union corresponding to any particular structural protein would then be determined by the specific character of the protein itself. Such a conception would correspond to that of specific catalyzers—the usual manner of conceiving these phenomena at present—the only difference being that the structural protein would itself play the part of catalyzer. Such a process would constitute a form of autocatalysis; the resemblance of growth to an autocatalytic process has in fact been emphasized by Loeb, Robertson, and others.[1] The structural peculiarities of such deposits, and hence the specific morphological characteristics of the cell, would be determined by the specific chemical characteristics of the constituent proteins, in a manner analogous to that by which the special form of a hæmoglobin crystal-aggregate is determined. And the special character of the structure thus laid down would determine the special character of the metabolism, and hence the special type of physiological activity exhibited by the cell. This last conclusion seems inevitable, since the source of this activity is metabolism, which in all living systems is under the control of structure. In other words, the formation of a specific structure in the protoplasmic substratum will necessitate a correspondingly specific type of metabolism, since the nature and rate of the metabolic chemical reactions are controlled by the structural conditions present; the dependent physiological or functional manifestations must therefore also be specific.

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[1] *Cf.* J. Loeb, *Biochem. Zeitschr.*, 1906, Vol. 2, p. 41. T. B. Robertson, *Arch. f. Entwicklungsmech.*, 1908, Vol. 25, p. 581. Wfg. Ostwald: Roux's *Vorträge und*

We are thus led to conceive certain features of the organic formative process in a somewhat definite manner, which may be summarized briefly as follows: The specific characters of any animal or plant are determined ultimately by the specific characters of its structure-forming proteins. The developing germ or the growing organism synthesizes specific proteins, and these, since they determine the structural and hence the physiological peculiarities of the organism, form the basis of its special character as an organic species. Accordingly one of our most fundamental problems is to determine why the cell builds up proteins of its own specific type. The essential problems of heredity and reproduction center here. As we have seen, heredity is exemplified whenever one yeast-cell or bacterium gives rise to another; also whenever any cell grows and increases its living organized material. This increase in living material is indispensable for the continuance of the species, and for this reason we may characterize growth as the fundamental life-process, and the problem of growth in its most general aspect as identical with the problem of heredity. The factors of growth are the factors of heredity.

Aufsätze, Heft 5, 1908. Chodat made a similar suggestion for plant growth in 1905 (*cf.* D'Arcy Thompson: "Growth and Form," Cambridge Univ. Press, 1917, p. 132).

Such a conclusion directs our particular attention to the general nature of the conditions controlling growth.

Growth and development may be controlled to a greater or less degree by various artificial means; and much of experimental embryology is concerned with modifying the rate and character of either process. In this way many definite hereditary characters may be profoundly altered, or in some instances their appearance may be altogether suppressed. A simple and instructive instance is described by Loeb.[1] The sea-urchin egg will develop to the gastrula stage in a balanced solution of sodium, potassium, and calcium chloride; if in addition to these salts some sodium carbonate is present, the skeletal spicules may form and a pluteus larva develop, but not otherwise. The skeleton is an inherited character; its formation, however, is dependent upon the presence of sodium carbonate in the surrounding medium, as well as upon the organization of the germ; the necessary carbonate must be furnished to the germ from without, or the specific formative process is unable to take place. Such a result is not difficult to understand. Development, like growth, is a matter of metabolism, and primarily of constructive metabolism; hence it is influenced by any condition that influences metabolism; accordingly the presence or absence of food, oxygen, water, salts, vitamines, hormones, as well as the conditions of temperature, may each and all have determinative relations to the total process. It is significant, however, that the specific characters of the organism, those which, according to the present view, express the chemical specificity of its structural proteins, seem never to be essentially altered by such changes of condition, although their appearance may be prevented or the degree of their development modified. Whatever structural characters appear in development are those characteristic of the species; this statement may be qualified to the degree required to take into consideration the facts of mutation (these suggest that under exceptional conditions new structural proteins may be synthesized); but the essential fact which we wish to express is the tenacity with which the organism preserves its specificity. At least this specificity can be modified, if at all, only by gradual degrees; and any theory of growth or heredity must assign some definite basis for this characteristic conservatism. We have suggested above that this basis is the tendency of similarly constituted compounds to segregate in the formation of aggregates, and in this way to form structures which determine the direction of metabolism. But such an hypothesis explains only the *resemblance* of an organism to its parent; it does not indicate how the proliferative process itself is carried out. It appears, in fact, that two separate groups of problems are involved in the theory of heredity—the one relating to the conditions determining the resemblance to the parent stock, the other relating to the nature of the physiological mechanism by which the living substance (apart from its special nature) increases its quantity or *grows* at the expense of materials taken from the surroundings. Such growth is a physiological activity requiring the expenditure of energy, and it cannot be considered apart from its relation to the other physiological, *i. e.*, functional, activities of the organism. The nature of this latter relation now calls for special consideration.

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[1] J. Loeb, *Amer. Journ. Physiol.*, 1900, Vol. 3, p. 441.

The general significance of the normal functional activity of the living system as one of the chief factors in the formation of its characteristic structure, or in structure-forming metabolism generally, has been insufficiently regarded by writers on heredity. In general, any normally active tissue maintains itself or grows, while an inactive tissue remains stationary or undergoes regression, even if supplied with an abundance of oxygen and food-material; this last is well shown in a voluntary muscle whose innervation has been interrupted. It is clear that increased functional activity involves an increase of constructive as well as of destructive metabolism; and conversely the subnormal metabolism accompanying inactivity is associated with subnormal construction, which may even fall below destruction, with regression or atrophy as a result. The control which function exercises upon the building-up of living structure is seen perhaps most clearly in higher organisms, and especially in tissues like voluntary muscle, whose activity is intermittent and subject to much variation. In this case the effects of exercise in promoting growth and of disuse in causing regression are familiar to all; and essentially similar conditions are found in other tissues and organs. Functional hypertrophy following excessive activity, and regression or atrophy following prolonged inactivity, are both well-known phenomena; for example, compensatory hypertrophy in heart muscle is a frequent result of valvular lesions, and one kidney increases in size if the other is removed. In general it would appear that any physiological function can reach and preserve its highest perfection only through continual repetition; and this very condition implies that decline must follow inactivity if the latter is prolonged beyond a norm. And since every function has some organized structure as its correlate, the same considerations apply to whatever structures are concerned in the function in question. The modifications which the central nervous system undergoes in association with the process of learning afford instances of an essentially similar kind; practise facilitates the repetition of any complex voluntary action, *i. e.*, perfects the structural and other adjustments underlying the function;[1] while any accomplishment, intellectual or other, declines with disuse. These examples may suffice to illustrate the general principle under consideration. It seems clear that the physico-chemical mechanisms—whatever their nature may be—controlling functional activity are in some intimate relation to those determining growth. The above facts seem to imply that both classes of physiological processes are simultaneously and equally under the control of some more general set of conditions characteristic of living substance in general. We shall now consider this possibility in more detail.

Claude Bernard has pointed out how essential it is in any living system—if the system is to continue to exist—that there should be a relation of interdependence between the processes of destruction and of repair, of such a kind that any destructive or dissimilatory process sets in motion automatically the contrary process of repair.[2] All functional activity involves breakdown of organized material, and apparently some disintegration of cell-structure then always takes place, for protein metabolism is increased—even though slightly under good nutritive conditions—by increased muscular work.[1] Hence for normal regulation of cell-activity it is essential that a compensatory or constructive series of processes should be aroused into action by the same conditions that stimulate or call forth the destructive or energy-yielding activity. "Functional breakdown in living material is itself the precursor and instigator of the renovation accomplished by the formative process, which works silently and obscurely in the interior of the tissue" (*i. e.*, without evident external manifestation, in contrast to the destructive process). "Losses are thus repaired as rapidly as they are caused, and since equilibrium tends to re-establish itself as soon as it is destroyed, the normal composition of the living body is maintained."[2] Bernard also recognizes that this process of restitution involves not only a chemical synthesis, but also a morphological synthesis, *i. e.*, a rebuilding of organized structure. The constructive and the destructive processes are inseparable; synthesis is life, whether during rest or activity. Hence the rate of construction must be regarded as ultimately under the same kind of control as the rate of destruction, even though the latter is more obviously subject to modification under the usual conditions of life (as in stimulation, voluntary action, etc.).[1] As instances of the initiation of organic construction by conditions whose primary effect is to stimulate, *i. e.*, to *increase* the energy-yielding dissimilation, Bernard cites the awakening of dormant germs or hibernating animals by rise of temperature or other stimulating condition;[2] the resumption of growth and other processes involving increased assimilation illustrates the constant, though it may be indirect, nature of the interconnection. An apposite present day-illustration of the same phenomenon would be the initiation of development in unfertilized eggs by a temporary cytolytic action. But one does not need to search for instances; the reciprocal interdependence of assimilation and dissimilation is seen everywhere in organisms; how essential this relation is for the preservation of life appears, for example, in the general fact that in all animals increased muscular or other activity hastens the onset of *hunger*, *i. e.*, of the condition necessary for supplying the raw material for construction. The maintenance of a balance between the two kinds of metabolic processes constitutes probably the most fundamental of the various types of organic regulation.

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[1] This is the basis of the phenomenon of *memory*. Hering has discussed briefly the relations between memory and heredity in his well-known address on "Memory as a General Function of Organized Matter," Vienna Academy, 1870; English translation by Open Court Publishing Company, Chicago, 1897.

[2] "Leçons sur les phénomènes de la vie," Vol. 1, Chapter 3.

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[1] It is now amply demonstrated that increased muscular work in higher animals leads to little or no increase in breakdown of protein (as indicated by N-excretion), provided the non-nitrogenous food-constituents are sufficient in quantity, especially the carbohydrates. If the food contains sufficient protein for maintenance, but carbohydrate and fat are deficient, there may be a considerable increase in N-excretion, but typically not enough to account for the increased energy-production on the basis of oxidation of protein; in this case the surplus of energy comes from the non-nitrogenous reserves of the organism. Carbohydrate is especially effective as a protein-sparer, a fact indicating that in the construction of protein it plays an essential part. It is also the chief source of muscular energy; and the fact that vigorous muscular work, involving active consumption of sugar, is the chief condition for the normal growth of the tissue, shows that the energy required for this growth—*i. e.* for the chemical and structural syntheses involved—is derived from the oxidation of sugar. The possible metabolic changes concerned in this process cannot be considered in an article like the present. But that carbohydrate is essential for the assimilation of amino-acids and other nitrogen compounds in both animals and plants is indicated by a large body of recent and older investigation. Thus for the assimilation of amino-acids by yeast and moulds sugar is indispensible (*cf.* the series of papers by F. Ehrlich, *Biochem. Zeitschr.*, 1906, Vol. 1, p. 8; 1908, Vol. 8, p. 438; 1909, Vol. 18, p. 391; 1911, Vol. 36, p. 477); similarly in higher plants the synthesis of protein from amides in germination requires the presence of carbohydrates (for a brief summary of the facts *cf.* Jost's "Physiology of Plants," p. 175). The work of Loewi, Lüthje and others has shown the great importance of carbohydrates in the synthesis of protein from amino-acids in higher animals; there is also clear evidence that derangement of carbohydrate metabolism (*e. g.*, in pancreatic or other diabetes) interferes very directly with the synthesis of protein (*cf.* Chapter 9 of Cathcart's "Physiology of Protein Metabolism" for a general review of this subject and literature).

[2] "Leçons sur les phénomènes de la vie," Vol. 1, p. 127.

There is no doubt that a general regulatory condition of this kind exists in all organisms; the problem is to determine the essential physico-chemical conditions upon which it depends. We must regard the living system primarily as one in which the synthesis of both chemical substance and organized structure is controlled by functional activity. And during the growth and development of the system, *i. e.*, at the periods when synthetic activity predominates, a similar dependence of formative activity on function must exist. This is the kind of relation emphasized recently by Child, when he describes the developing organism not as being first constructed and then functioning, after the manner of a machine, but as constructing itself *by* functioning.[1] Growth and development are peculiar in that specific construction overbalances destruction, and that the synthesized material, as it accumulates, adopts a definite organization. But this accumulation of structure is itself the expression or result of active functioning, in which energy is freed, just as growth is such an expression in a muscle which has been exercised. Apparently we must conclude that part of this energy is expended in the work of synthesizing and arranging the specific structural material of the organism or cell. We have already seen that this specific material consists essentially of protein. We are thus brought back to the question: what are the conditions under which protein is synthesized in the living cell and deposited as structure?

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[1] Bernard gives direct experimental evidence of the identity of the conditions controlling growth with those controlling stimulation by showing that growth (*e. g.* in seedlings) may be anæsthetized under the same conditions as the different forms of irritability. *Cf.* his chapter on Irritability, Leçons, Vol I, p. 267.

[2] *Cf.* Leçons, Vol. I, Chapter 2, p. 110.

No very definite or certain answer can be given at present to this question. But we seem to be in a position to rule out certain possibilities, and perhaps to affirm others. First we must note more particularly the significance of the long recognized fact that many vital syntheses require the addition of energy to the synthesized compounds; this is seen, for instance, in the formation of fats from proteins and carbohydrates. Now since such syntheses, where compounds of higher chemical potential are built up from those of lower, take place continually in all cells, it appears highly probable that their conditions are also the main conditions of synthesis in general, and that a subordinate importance is to be attached to the purely enzymatic type of synthesis. Both experience and theory show that the latter is limited to the formation of compounds in which little change of energy accompanies the transformation;[2] hence it is plainly insufficient to meet the normal requirements of constructive metabolism. Some kind of mechanism would seem to be indicated in which energy derived from oxidation or other chemical source is applied to perform the work of chemical and structural synthesis. Enzymes may facilitate or direct certain kinds of combinations, and in this way they may be important as accessory factors in the constructive process; but the essential controlling factors are evidently of a more active, *i. e.*, work-performing, kind. And since these factors vary in their activity with function, it follows that the conditions controlling the degree and rate of functional activity—*i. e.*, generally speaking, the conditions of stimulation—must at the same time be the conditions controlling the specific constructive processes.[1] It is true that construction does not always run parallel with destruction, the rate of which may often temporarily exceed that of repair; and at times nutritive or other conditions may render complete replacement impossible; or at other times construction may preponderate, as in growth. Nevertheless an interdependence of the kind indicated unquestionably does exist; and apparently we must infer that part of the energy freed in the oxidation (or other energy-yielding decomposition) which performs the work of function is applied, in some manner as yet unknown, to build up the material required for maintenance or further growth.

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[1] C. M. Child, "Individuality in Organisms." University of Chicago Press, 1915, p. 16.

[2] *Cf.* Höber's "Physikalische Chemie der Zelle und der Gewebe," 1914, p. 677.

If we adopt this general hypothesis, we must reject as entirely insufficient the conception of growth as being analogous to a process of crystallization, or as being determined by syntheses of the enzymatic kind; and we are led to look for some other type of process in which the formation and deposition of structural material is controlled by energy set free in chemical change. This process must be capable of variation in rate, of interruption and renewal, and of reversal,—if it is to correspond to such features of organic growth as the variation of the latter with the conditions (*e. g.*, temperature or the supply of food and oxygen), its dependence on function, and the possibility of regression. Further, it must be a process not necessarily peculiar to living organisms, although apparently taking place under especially favorable conditions in these systems, and it must be able to effect either chemical decompositions or syntheses. All of these peculiarities seem to point to some physico-chemical process of the general nature of electrolysis as underlying the synthetic activity of organisms. In other words the possibility presents itself that electrosynthesis is the chief method of construction in the living system.

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[1] This is indicated by Bernard's already cited observation that anæsthetics arrest growth-processes reversibly in the same manner as they inhibit stimulation or other forms of functional activity,—a fact suggesting that physico-chemical changes of the same nature control both growth and the response to stimulation. If this is true, it seems probable that the structures primarily concerned in stimulation are also those primarily concerned in construction, *i. e.*, the site or *locus* of both constructive and destructive processes is the same, the two representing reverse phases of the same process. On such a view the idea that special regions of the cell (*e. g.*, the nucleus) are the exclusive seat of syntheses would have to be abandoned. There is, however, much evidence that the nucleus is necessary for the continuance of synthetic processes; possibly it gives rise to certain substances which are required for the maintenance of the structures more immediately concerned in the specific syntheses.

The main physiological facts which appear to me to favor this hypothesis are briefly as follows. All functional activity is associated with the formation of electrical circuits between different regions of the cell or organism. The currents of these bioelectric circuits are in many cases sufficiently intense to produce marked physiological effects upon other cells or tissues (stimulation, etc.), and presumably they have similar effects upon other regions of the same cell; the transmission of the effects of local excitation appears in fact to be due to an action of this kind. In general we observe vital functions to be profoundly influenced—accelerated, inhibited, or initiated—by electrical influence; and since function involves specific construction, the constructive process must be subject to similar influence. Experimental data upon the influence of the electric current on growth processes are as yet comparatively few; but galvanotropism is well known in plants, and with properly devised experimentation could probably be shown to be widespread. The control of growth processes by the electric current offers in fact a largely untouched field of investigation, which would probably yield results not only of great theoretical interest but of practical importance as well (*e. g.*, as possibly affording a means of controlling malignant growths, etc.).

But perhaps the clearest indications that the organic formative processes are under the control of electrical conditions are seen in the striking resemblances which certain types of electrolytic deposit show, both in their structural character and in the conditions influencing their formation, to the structures arising by normal growth in organisms. The two types of phenomena exhibit many close and, as it seems to me, highly significant parallels. Inorganic structures resembling vegetative growths are seen not only in the formation of lead or tin "trees" from metallic zinc immersed in solutions of salts of these metals, but they are shown in an especially striking form in those cases where the local process of electrolysis gives rise to precipitates which form coherent membranes or otherwise exhibit colloidal character. In a recent paper I have described the structure and conditions of formation of such precipitation-structures in some detail, and have discussed the reasons for their resemblance to organic growths.[1] In solutions of potassium ferricyanide, especially those containing egg-albumin or other protective colloid, pieces of metallic iron, zinc, or copper produce characteristic filamentous or quasi-cellular outgrowths, consisting of precipitation-membranes of the ferricyanide of the metal used, which resemble strongly certain definite organic types of growth like mould-hyphæ. These structures grow out into the solution from the anodic regions of the metallic surface; hence their formation may be accelerated, retarded, or suppressed at will by varying the character of the local circuits determining the rate at which the ions of the metal enter solution. A region of (*e. g.*), iron which is actively forming precipitation-filaments will at once cease this action if it is rendered the cathode in another intercepting local circuit,—*e. g.*, by the contact of zinc or other baser metal at a neighboring point; or conversely it may be rendered still more active—*i. e.*, more strongly anodic—by increasing the intensity of the local circuit in which it acts as anode,—*e. g.*, by the adjacent contact of a nobler metal (*e. g.*, platinum) or carbon.[2] It is especially to be noted that the relations between the different electrode-regions of such local circuits are *reciprocal*, as regards the character of the chemical changes there taking place; this is inevitable, since in general the electrochemical processes at any anode are the reverse of those at the cathode. Hence the formation of filaments at one region of the metallic surface appears to have the effect of *inhibiting* their formation at another adjoining region. A similar reciprocality of influence is especially characteristic of physiological processes, such as excitation and inhibition, *e. g.*, in the central nervous system; and it is also well known to be characteristic of various processes of growth and regeneration in both animals and plants.[1] This is why (for example) cutting off a tubularian head enables an adjoining region of the stem to form a new hydranth; the region where the new growth takes place has been removed (by the operation) from the inhibiting influence of the original hydranth. Similarly a short piece of iron wire which is in contact with a piece of zinc will not form filaments in ferricyanide solution until the zinc is detached or otherwise rendered inactive. Cutting away the zinc thus initiates the development of filaments from the iron;[2] the structure-forming process had previously been inhibited by the activity at the zinc, which on account of its greater tendency to send ions into solution alone forms filaments while the two metals are in contact. To express the matter biologically: the zinc seems actively to appropriate the available structure-forming material (ferricyanide), and in so doing prevents the iron from utilizing this material to form filaments. Similarly the hydranth, with its higher rate of metabolism, acts as the chief structure-forming region in the tubularian, and inhibits structure-formation of the same kind in its vicinity.[3] It corresponds, in this sense, to the anodal metal in the local electrical couple of zinc and iron. The growth-initiating consequences following physiological isolation—to use Child's concise and illuminating expression[4]—may thus be instructively simulated by means of an inorganic model of this kind.

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[1] BIOLOGICAL BULLETIN, 1917, Vol. 33, p. 135.

[2] *Loc. cit.*, p. 148.

These and similar parallels appear to indicate that *the same type of process* is concerned in the structure-formation in the two kinds of system, otherwise so entirely unlike in character.[5] If this is true, the transmission of the structure-forming or growth-inhibiting influence is in both cases due to the formation of electrical circuits between the influenced and the influencing regions. In their morphological details and in their chemical composition the structures formed are, needless to say, widely different in the living system and in its inorganic model, although in certain peculiarities of physico-chemical constitution—especially the cellular and filamentous character of the formations and the part played in both by semipermeable membranes—the precipitation-structures and the living systems show significant resemblances. A precipitation-structure of a definite chemical composition even shows a certain morphological specificity, *i. e.*, the structures formed from zinc are characteristic in their appearance and different from those formed from iron or copper. We may say that under the influence of the metal the ferricyanide of the solution is transformed into structure of a definite kind. And this structure, once formed, becomes the condition of formation of other similar structure.[1]

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[1] *Loc. cit.*, pp. 156, 163.

[2] *Loc. cit.*, pp. 152 *seq.*

[3] This is an example of the dominance or control of formative processes by those regions having the highest rate of metabolism: *cf.* Child's "Senescence and Rejuvenescence," Chapter 9, p. 210; also "Individuality in Organisms," Chapter 5.

[4] *Loc. cit.*

[5] It is a question whether an electric current passing between any semi-permeable water-insoluble phase and the adjoining aqueous solution can do so otherwise than by a process of ionization (or deionization) at the boundary, *i. e.*, by a process

To complete the resemblance to a growing plant-filament or other proliferating living system, the structures thus formed ought to be capable, after isolation, of forming more structure of the same kind. This is of course not the case with the precipitation-filaments, taken by themselves, since the connection with the metal is essential; but the difference may be regarded as due mainly to incidental conditions. The growing system is in fact not constituted by the filaments *alone*, but by the combination of filaments, metal and solution; the formative process depends upon the interaction of all three. Something analogous may be said to be true of the growing organism; in a spore placed in a

involving electrolysis. This is what takes place in the passage of a current between a metallic surface and a solution; in this case the addition or abstraction of electric charges to or from substances present at the boundary is what forms the essential condition of the electrolysis there taking place. The cell-surface is similarly water-insoluble, and semi-permeability is characteristic; yet it allows the passage of the electric current, although with a somewhat high and variable resistance. The facts of polar stimulation, polar disintegration, etc., indicate that where the current *enters* the cell-surface it produces different chemical effects from where it *leaves*, just as in electrolysis at metallic surfaces.

[1] *Cf.* the description of the mode of formation of precipitation-filaments, *loc. cit.*, pp. 143–144.

culture-medium the structure of the germ, its chemical composition, and the chemical composition of the culture-medium, are all equally essential factors in the resulting growth-process. The main difference is that each living cell, as soon as formed, is capable of acting as a similar center of transformation when transferred to another culture-medium; *i. e.*, *all* of the necessary parts of the proliferating system are multiplied equally; and so far we have been unable to produce any artificial systems having such properties. If we were to succeed in doing so, it is probable that such systems would exhibit a much closer resemblance to living organisms than any of the inorganic models hitherto used for comparisons of the above kind.

Obviously these fundamental resemblances between the two types of system under comparison do not preclude infinite differences in the details of structure, chemical composition, and activity; but I am at present insisting upon the resemblances because of the desirability of determining the *class* to which the organic formative processes belong. The characteristic plasticity and responsiveness of living matter undoubtedly depend upon the fundamental features of its physico-chemical constitution. Starting with living material of this peculiar type of self-regulating structure and chemical composition, the developmental process has in the course of time become so evolved and perfected that it now builds up with unfailing regularity the most complex of organisms from the food and other materials furnished to the germ from the surroundings. But the possibility of this development has depended upon certain general pecularities of physico-chemical constitution present from the beginning in the living formative substance itself; and my aim in the present and preceding papers is to indicate what seem to me the most essential of these peculiarities.

The case of higher organisms presents numerous problems of a more special kind, and most investigators in the field of heredity have given their chief attention to these problems. It seems clear that in these organisms other and more special mechanisms of hereditary coördination and control have been superposed upon the elementary physico-chemical mechanism which conditions the fundamental proliferative activity. The fact that in higher animals particular cell-structures like chromosomes may become essential regulatory or determinative factors, controlling the detailed character of the developmental proliferation, is in no sense inconsistent with the general point of view here presented. All protoplasmic structures as they originate must influence formative metabolism, as has been so ably pointed out by Child; and there is every evidence that the chromosomes have a peculiarly intimate relation to the distribution of form-determining factors. Recognition of the part played by hormones in development is also consistent with the present view. What we are now attempting, however, is not to define the special mechanisms governing the course of development in the higher metazoa, but to indicate the nature of the more general physico-chemical mechanism, common to all forms of living matter, which forms the basis of its characteristic self-conserving and proliferative properties. Any conception of the essential constitution of living matter must first of all take its constant and fundamental distinguishing peculiarities into account. Once the living material has come into existence, with such properties as these, it may serve as the basis for the development of diversity and complexity of all kinds, as has in fact occurred in the course of evolution.

APPETITES AND AVERSIONS AS CONSTITUENTS OF INSTINCTS.

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GENERAL ACCOUNT OF APPETITE AND AVERSION.

The overt behavior of adult animals occurs largely in rather definite chains and cycles, and it has been held that these are merely chain reflexes. Many years of study of the behavior of animals—studies especially of the blond ring-dove (*Turtur risorius*) and other pigeons—have convinced me that instinctive behavior does not consist of mere chain reflexes; it involves other factors which it is the purpose of this article to describe. I do not deny that innate chain reflexes constitute a considerable part of the instinctive equipment of doves. Indeed, I think it probable that some of the dove's instincts include an element which is even a tropism as described by Loeb. But with few if any exceptions among the instincts of doves, this reflex action constitutes only a part of each instinct in which it is present. Each instinct involves an element of appetite, or aversion, or both.

An appetite (or appetence, if this term may be used with purely behavioristic meaning), so far as externally observable, is a state of agitation which continues so long as a certain stimulus, which may be called the appeted stimulus, is absent. When the appeted stimulus is at length received it stimulates a consummatory reaction, after which the appetitive behavior ceases and is succeeced by a state of relative rest.

An aversion (example 7, p. 100) is a state of agitation which continues so long as a certain stimulus, referred to as the disturbing stimulus, is present; but which ceases, being replaced by a state of relative rest, when that stimulus has ceased to act on the sense-organs.

The state of agitation, in either appetite or aversion, is exhibited externally by increased muscular tension; by static and phasic contractions of many skeletal and dermal muscles, giving rise to bodily attitudes and gestures which are easily recognized signs or "expressions" of appetite or of aversion; by restlessness; by activity, in extreme cases violent activity; and by "varied effort" (Lloyd Morgan, '96, 7, 122, 154; Stout, '07, 261, 267).

In the theoretically simplest case, which I think we may observe in doves to some extent, these states bring about the appeted situation in a simple mechanical manner. The organism is disturbed, actively moving, in one situation, but quiet and inactive in another; hence it tends to move out of the first situation and to remain in the second, obeying essentially the same law as is seen in the physical laboratory when sand or lycopodium powder on a sounding body leaves the antinodes and comes to rest in the nodes.

But pigeons seldom are guided in so simple a manner. Their behavior involves other factors which must be described in connection with appetite and aversion.

An appetite is accompanied by a certain *readiness to act.* When most fully predetermined, this has the form of a chain reflex. But in the case of most supposedly innate chain reflexes, the reactions of the beginning or middle part of the series are not innate, or not completely innate, but must be learned by trial. The end action of the series, the consummatory action, is always innate. One evidence of this is the fact that in the first[1] manifestation (also, in some cases, in later performances) of many instincts, the animal begins with an *incipient consummatory action*, although the appeted stimulus, which is the adequate stimulus of that consummatory action, has not yet been received. I speak of an incipient "action" rather than "reaction," because it seems clearly wrong to speak of a "reaction" to a stimulus which has not yet been received. The stimulus in question is obtained only after a course of appetitive, trial-and-error behavior. When at last this stimulus is obtained, the consummatory reaction takes place completely, no longer incipiently. Then the appetitive behavior ceases; in common speech we say the animal is "satisfied."

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[1] To see the appetitive nature of an instinct, it is necessary in some cases to observe an individual animal carefully during its first performance of the act in question. But the performance may be so quick that the observer is quite unable to analyze it. Analysis may be aided by preventing the animal from attaining the consummatory situation for a time, so that the appetitive phase is prolonged, as it were magnified. My cripple dove (example 5, p. 99) afforded just this aid to analysis. The literature is full of reports of instinctive behavior which might well be further analyzed. Consider for example the case of the young moorhen cited by Lloyd Morgan ('96, 63) which had never previously dived, but on being suddenly frightened by a puppy, dived like a flash. That act was too quick for us to analyze it. But if we could successfully impede the diving of a young moorhen so as to prolong the phases of the act, I think it probable that we should find an appetite for the consummatory situation (that of being under water) and a restless striving until it is attained; and that some details in the series of actions, details which in a normal dive are very sure to be hit upon by accident, are not innately predetermined. When one sees the first performance of an instinctive act take place very quickly and with apparent perfection, this does not prove that there is an innate chain reflex determining every detail of the act.

One may observe all gradations between a true reflex and a mere readiness to act, mere facilitation. Thus, in the dove, a stimulus from food in the crop may cause the parent to vomit the food or to feed it to young: there are all gradations from an immediate crop-reflex, in which the food is vomited upon the ground, through intermediate cases in which the parent is much disturbed by the food in his crop, but appetitively seeks the young and induces them to take the food; to other cases is which the parent is only ready to feed the young if importuned by them; and finally to cases in which the stimulus from the crop does not even cause facilitation, and the parent does not disgorge the food at all, even if importuned by the young.

While an appetite is accompanied by readiness for certain actions, it may be accompanied by a distinct *unreadiness* for certain other actions, and this is an important factor in some forms of behavior. It is altogether probable that this unreadiness is due in some cases to the fact that the activity of certain neurones *inhibits* the activity of certain other neurones. It is now well-known, too, that unreadiness may be due to the condition of the internal secretions. And the mutual exclusion of certain forms of instinctive behavior is inevitable, due to the incompatibility (Washburn, '16) of their motor components.

Unreadiness may be accompanied by aversion, and vice versa; but either of these may occur without the evident presence of the other. An aversion is sometimes accompanied by an innately determined reaction adapted to getting rid of the disturbing stimulus, or—this point is of special interest—by two alternative reactions which are tried and interchanged repeatedly until the disturbing stimulus is got rid of (see example 7, page 100).

The escape from a disturbing situation or the attainment of an appeted one is accomplished, in case of some instincts, far more surely and more rapidly after one or more experiences. In the first performance of an appetitive action, the bird makes a first trial; if this fails to bring the appeted stimulus he remains agitated and active, and makes a second trial, which differs more or less from the first; if this fails to bring the appeted stimulus he remains still active and makes a third trial; and so on until at last the appeted stimulus is received, the consummatory reaction follows, and then the bird comes to rest. In later experience with the same situation, the modes of behavior which were followed immediately by the appeted stimulus and consummatory reaction are repeated; those which were not so followed tend to drop out.

If a young bird be kept experimentally where it cannot obtain the normal stimulus of a certain consummatory reaction, it may vent that reaction upon an abnormal or inadequate stimulus, and show some satisfaction in doing so; but if the bird be allowed at first, or even later, to obtain the normal stimulus, it will be thereafter very unwilling to accept the abnormal stimulus. That this is true of the sex instinct has been shown in a former article (Craig, '14). It is true also of the appetite for a nest. Thus a female dove which has never had a nest, nor material to build one, lays eggs readily on the floor; but a dove that has had long experience with nests will withhold her egg if no nest is obtainable. The male dove similarly, if he has never had a nest, goes through the brooding behavior on the floor; but an experienced male is unwilling to do so, and shows extreme anxiety to find a nest. These examples illustrate the fact that the bird must *learn* to obtain the adequate stimulus for a complete consummatory reaction, and thus to satisfy its own appetites.

There is often a struggle between two appetites, as when a bird hesitates, and it may hesitate for a long time, between going on the nest to incubate and going away to join the flock, eat, etc. By watching the bird one can predict which line of behavior it will follow, for each appetite is distinguished by its own expressive signs (consisting partly of the incipient consummatory action), and one can see which appetite is gaining control of the organism.

These outward expressions of appetite are signs of physiological states which are but little known. Since my own observations have been on external behavior only, I say little about the internal states. They are probably exceedingly complex and numerous and similar to the physiological states which in the human organism are concomitants of appetites,[1] emotions, desires. They doubtless include stimulations from interoceptors and proprioceptors; perhaps automatic action of nerve centers; perhaps readiness or unreadiness of neurones to conduct. It is known that some of the periodic appetites are coincident with profound physiological changes. Thus Gerhartz ('14) found that during the incubation period in the domestic fowl the metabolism of the body as a whole is at a low ebb. In some cases a stimulus from the environment is the immediate excitant of an appetite; especially, stimulation of a distance-receptor may arouse appetite for a contact stimulus, as when the sight of food arouses appetite for the taste of it. But probably in every case appetite is dependent upon physiological factors. And in many cases the rise of appetite is due to internal causes which are highly independent of environmental conditions, and even extremely resistant to environmental interference.

Appetitive behavior in vertebrates is evidently a higher development of what Jennings ('06, p. 309) calls the positive reaction in lower organisms; aversive behavior in vertebrates corresponds to what Jennings (p. 301) calls negative reactions.

The attempt to distinguish between instinct and appetite, as in Baldwin's Dictionary ('01), is not justified by the facts of behavior. Baldwin says : "Appetite is distinguished from instinct in that it shows itself at first in connection with the life of the organism itself, and does not wait for an external stimulus, but appears and craves satisfaction." These characteristics, here ascribed to appetite, are the very ones which I have observed in the instinctive behavior of pigeons. The instincts of pigeons satisfy Baldwin's further description of appetite in that each appears first as a "state of vague unrest" involving especially "the organs by which the gratification is to be secured"; and "a complex state of tension of all the motor . . . elements whenever the appetite is aroused either (*a*) by the direct organic condition of need, or (*b*) indirectly through the presence or memory of the object." This last point is illustrated, *e. g.*, by doves learning to drink (example 1, page 97), in whom the sight of the water-dish at a distance aroused the drinking actions by associative memory. I have observed appetitive behavior as Baldwin describes it in nearly all the instinctive activities of doves, and I think that sufficient observation will reveal it in all their instincts.

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[1] Hunger furnishes a typical case of appetitive behavior (Carlson, '16; Ellis '10, 198–199). Carlson makes a distinction between hunger and appetite. The distinction he finds is certainly real, but the use of words is unfortunate, for hunger is clearly one kind of appetite.

The most thorough attempt to distinguish instincts from appetites and to show the logical consequences of such distinction, in all the literature to which I have access, is in an old article by Professor Bowen ('46). This article is still worth study, to suggest the conclusions to which one is logically led if he denies that instincts contain any element of appetite. These conclusions, taken almost literally from Bowen, may be summarized as follows: (1) (P. 95) "If the name of instinct be denied to these original and simple preferences [appetites] and aversions, there will appear good reason to doubt whether man is ever governed by instinct, whether all his actions are not reducible to passion, appetite, and reason." (P. 115) The "passions" of man can not be concomitants of instinct. (2) (P. 117) "Instinct is not a free and conscious power of the animal itself. It is, if we may so speak, a foreign agency, which enters not into the individuality of the brute." (P. 118) Instinct "has no effect on the rest of their conduct, which is governed by their own individuality." (3) Bowen contends with logical consistency that if instinct contains no appetitive factor, the ends toward which instincts work, as seen by an observer, are not ends for the agent; that therefore the agent has no power to make the instinctive behavior more effective. In short, instinctive behavior is not susceptible of improvement by intelligence. (4)

Bowen concludes that the intellect and the "passions" of man are not products of evolution. (5) It may be added that even Bowen, strive as he did to separate appetite from instinct, was compelled to admit that the attempt at such separation leads one into difficulties and disputed cases. In contravention of Bowen's conclusions I contend: (1) That much of human behavior is instinctive. (2) That Bowen's description of instinct as "a foreign agency, which enters not into the individuality" is true of reflex action, such as coughing or sneezing, but is not true of instinctive behavior, which is extremely different from such mere reflexes. (For a fuller statement on this point, see below, page 106. See also Hobhouse, '15, 98–99.) (3) That, of the useful results toward which instincts tend, some, not all, are ends for the agent. For they are the objects of appetites, and the animal strives and learns to attain them. (4) That human conative behavior evolved from the instinctive appetitive behavior of lower animals.

In another article I hope to publish soon a further discussion of the literature.

EXAMPLES.

1. The case of doves learning to drink, as described in detail in a former article (Craig, '12), illustrates appetite. The observed appetitive behavior was aroused by stimulation of distance-receptors, such as the sight of the water-dish being brought to the cage, and of the man bringing it; these acted as appetizers. Each dove, as soon as it had learned to associate such stimuli with the drinking situation, responded to these stimuli by making drinking movements (incipient consummatory action) at once without going to the water dish. The first drinking movements failing to bring water, the dove repeated these movements again and again, sometimes walking a few steps, sometimes turning round, until after many trials and many errors it did get its bill into the water, received the stimulus from water in the mouth (appeted stimulus), whereupon the drinking movements (consummatory reaction) were made not incipiently but completely, the water being swallowed, after which the bird rested and appeared satisfied.

2. A good example of appetitive behavior is seen in the way in

which a young male dove locates a nesting site for the first time. The first thing the observer sees is that the dove, while standing on his perch, spontaneously assumes the nest-calling attitude, his body tilted forward, head down, as if his neck and breast were already touching the hollow of a nest (incipient consummatory action), and in this attitude he sounds the nest-call. But he shows dissatisfaction, as if the bare perch were not a comfortable situation for this nest-dedicating attitude. He shifts about until he finds a corner which more or less fits his body while in the tilted posture; he is seldom satisfied with his first corner, but tries another and another. If now an appropriate nest-box or a ready-made nest is put into his cage, this inexperienced dove does not recognize it as a nest, but sooner or later he tries it, as he has tried all other places, for nest-calling, and in such trial the nest evidently gives him a strong and satisfying stimulation (the appeted stimulus) which no other situation has given him. In the nest his attitude becomes extreme; he abandons himself to an orgy of nest-calling (complete consummatory action), turning now this way and now that in the hollow, palpating the straws with his feet, wings, breast, neck, and beak, and rioting in the wealth of new, luxurious stimuli. He no longer wanders restlessly in search of new nesting situations, but remains satisfied with his present highly stimulating nest.

3. Fetching straws to the nest is apparently due to an appetite for building them into the nest. The dove has an innate tendency to pick up straws, and an innate tendency to build them into the nest (consummatory reaction); but it has apparently no innate tendency to carry a straw to the nest, no innate "chain" of reflexes. When an experienced bird finds a straw he seizes it repeatedly and toys with it, sometimes making movements resembling those by which he would build the straw into the nest. He seems thus to get up an appetite for building the straw in, and when this appetite is sufficiently aroused he flies to the nest, guided by associative memory, and performs the consummatory reaction completely. A young female, no. 70, which I observed picking up a straw for the first (?) time, on her 54th day, showed the lack of a "chain reflex." For she continued toying with the straw an excessively long time, not carrying it at all,

though she happened to be very near the nest. This was the more remarkable as she had a well-formed habit of going to the nest on all occasions. At length she did go to the nest with her straw, and made well-ordered movements to build it in.

4. The male and the female dove take regular turns in sitting on the eggs. The male is seized by the appetite for brooding about 8 or 9 A. M., and the female about 5 P. M., the state evidently being brought on in each case by physiological causes which are part of the daily physiological rhythm. When either one, *e. g.*, the female, comes to the side of the nest prepared to enter and sit, she already has somewhat the attitude of the sitting bird, the body sunk down on the legs and the feathers fluffed out (incipient consummatory action). If her sitting appetite be thwarted, as by her mate refusing to budge from his position, she shows restlessness and makes intelligent efforts to obtain possession of the nest. When at last her mate yields his place, she steps exultingly into the center of the nest and settles herself on the eggs with many movements indicative of satisfying emotion (complete consummatory reaction).

A broody hen of course illustrates the same principle.

5. It is an interesting fact, exhibited in a variety of instincts, that a young bird may make feints of performing actions which it has never yet performed. Thus the young dove makes feints of flying before it has ever flown. This was illustrated in a peculiarly instructive manner by one of my young doves, no. 46, which developed cripple wings and was unable to fly. When placed in a box with sides 3½ inches high it was just able to jump on the edge. Nevertheless, when its roosting instinct developed, it endeavored strenuously every evening to fly to the perch which was some inches above its head. It looked at the perch and aimed at it with perfect definiteness, opening its wings and making feints of flying. In the evolution of birds, there can be no doubt, flying developed gradually from jumping. The new movements of flying were gradually intercalated into the interval between the initial action, leaping from the ground, and the final action, landing again upon the feet. The young dove to this day shows *first* the incipient end action, aiming at the perch to be alighted on, and only after it has launched itself

toward this end situation does the "chain" of flight reactions take place.

6. In the pigeons the order of activities culminating in the sexual act is, first display, second billing, third copulation, with numerous details each finding a place in the succession. Yet the sexual tendency is mainfestly present from the beginning of the "chain," and the preliminary steps are directed, with much guidance by experience, toward securing the stimulation required for discharging the sexual reflex. In absence of the normal stimulus to the consummatory reaction, the instinct manifests itself in marked appetitive behavior, and, especially in inexperienced birds (Craig, '14), in those imperfect consummatory reactions known as perversions and auto-erotic phenomena. The behavior of the sexual appetite is now so well known that it may be cited as the type of appetitive behavior; and to readers who are familiar with modern analyses of the sex instinct I may make my whole article clearest by saying that all the appetitive mechanisms I have mentioned, and I believe all the instincts of the dove, behave in the same manner as that of sex, in regard to appetitive manifestations and anticipation of the consummatory reaction.

7. I shall take space to describe only one example of aversion—the so-called jealousy of the male dove, which is manifested especially in the early days of the brood cycle before the eggs are laid. At this time the male has an aversion to seeing his mate in proximity to any other dove. The sight of another dove near his mate is an "original annoyer" (Thorndike, '13, Chap. IX.). If the male sees another dove near his mate, he follows *either of two* courses of action; namely, (*a*) attacking the intruder, with real pugnacity; (*b*) driving his mate, gently, not pugnaciously, away from the intruder. When he has succeeded either in conquering the stranger and getting rid of him, or in driving his mate away from the stranger, so that he has got rid of the disturbing sight of another dove in presence of his mate, his agitation ceases. If we prevent him from being successful with either of these methods, as, by confining the pair of doves in one cage and the third dove in plain sight in a contiguous cage, then he will continue indefinitely to try both methods. If we leave all three

doves free in one pen, the mated male will try the mettle of the intruder and conquer him if he can; if he fails, he will turn all his energies into an effort to drive his mate away from the intruder. Or if in former experiences he has learned to gage this individual intruder, if he conquered him before he will promptly attack him now, but if defeated by him before he will now choose the alternative of driving his mate away. In sum, the instinctive aversion impels the dove to thoroughly intelligent efforts to get rid of the disturbing situation.

8. In some cases the seeking of a certain situation involves both appetences and aversions in considerable number. Thus, when the day draws to a close, each dove seeks as its roosting-place a perch that is high up, with free space both below it and above it, with no enemies near, with friendly companions by its side, but these companions not too close, not touching (except in certain cases of mate, nest-mate, or parent). The endeavor to achieve this complex situation, to secure the appeted stimuli and to avoid the disturbing ones, keeps the birds busy every evening, often for an hour or more.

CYCLES.

Instinctive activity runs in cycles. The type cycle, as it were a composite photograph representing all such cycles, would show four phases as follows.

Phase I.—Absence of a certain stimulus. Physiological state of appetite for that stimulus. Restlessness, varied movements, effort, search. Incipient consummatory action.

Phase II.—Reception of the appeted stimulus. Consummatory reaction in response to that stimulus. State of satisfaction. No restlessness nor search.

Phase III.—Surfeit of the said stimulus, which has now become a disturbing stimulus. State of aversion. Restlessness, trial, effort, directed toward getting rid of the stimulus.

Phase IV.—Freedom from the said stimulus. Physiological state of rest. Inactivity of the tendencies which were active in Phases I., II., III.

Some forms of behavior show all four phases clearly. The following are examples.

Sex.—(Phase I.) The dove, either the male or the female, shows sexual appetite and invites the mate to sexual activity. Gradually they lead up to (Phase II.) the consummatory sexual act. (Phase III.) After the sexual act, in some cases one bird shows marked aversion, *e. g.*, by striking at the mate. Either the male or the female may show aversion. In some species, signs of aversion after the sexual act seem to be a normal and regular occurrence. In other species they are shown only by a bird whose mate, having failed of satisfaction, invites to further sexual activity. (Phase IV.) The pair usually become sexually indifferent for a considerable time after each copulation.

Brooding.—(Phase I.) The dove shows the brooding appetence, goes to the nest, and, if need be, struggles to obtain possession of it. (Phase II.) It sits throughout its customary perod, during which it often resists efforts of the mate to relieve it. (Phase III.) At the end of this period, in contrast, it comes off at a slight sign from the mate, runs about, flaps its wings, and thus shows its joy in being off. This may be interpreted as a sort of mild aversion for the nest. (Phase IV.) It goes away and becomes temporarily indifferent to the nest.

In other cases, one or other of the phases is not clearly present, so that there are various sorts of incomplete cycles, such as the following.

(*a*) When the bird shows appetitive behavior but fails to obtain the appeted stimulus, the appetite sometimes disappears, due to fatigue or to drainage of energy into other channels; in which case, Phase II. is not attained.

But many instinctive appetites are so persistent that if they do not attain the normal appeted stimulus they make connection with some abnormal stimulus (see page 94); to this the consummatory reaction takes place, the tension of the appetite is relieved, its energy discharged, and the organism shows satisfaction. This is of course *compensation*, in the sense in which that word is used in psychiatry. But the abnormal stimulus is usually inadequate or incomplete, the relief or discharge is imperfect, the satisfaction is marred by the fact that some of the constituent elements of the appetite, failing to receive their appeted stimuli, are still in Phase I. and abnormally active, while at the same time other elements have already reached Phase III., aversion.

(*b*) Some forms of behavior consist of appetite and satisfaction which are not, in ordinary cases, followed by any distinct aversion. For example, the drinking cycle shows clearly: (Phase I.) appetite for water; (Phase II.) the drinking reaction, with expression of satisfaction; (Phase IV.) indifference. The dove when it finishes drinking shows no distinct sign of aversion (Phase III.) except withdrawing the bill from the water. But if the observer takes this dove then gently in the hand and re-submerges its bill in the water, it shows marked aversion, struggling to withdraw the bill and to shake the water out of it.

(*c*) On the other hand it may seem that there are some forms of behavior, *e. g.*, fear, in which Phases I. and II. are lacking; that there is no appetite for the fear stimuli and no satisfaction in them; that when the slightest of these stimuli is received it at once arouses (Phase III.) aversive behavior. Yet it is an interesting fact that even in these cases a slight degree of appetite and satisfaction may be present. Children seek and enjoy a little fear. A dove, when it hears the alarm cry from other doves, at once endeavors to see the alarming object. Even pain is (in man) to some degree, sought and enjoyed.

In actual life the cycles and phases of cycles are multiplied and overlapped in very complex ways.

For example, when a certain satisfaction has been attained, this, instead of leading at once to a state of surfeit and aversion, may lead to further appetite, which leads to a second satisfaction, and so on. Thus Phase I. and Phase II. continue to alternate, constituting a "circular reaction" (Baldwin). I have seen a pair of house sparrows copulate thirteen times in immediate succession, and know by the sound of their voices that I did not see the beginning of the series. In many cases such circular reaction serves to rouse the organism to a high state of appetite and readiness for action.

Smaller cycles are superposed upon larger ones. For example, when a female bird is building a nest, so long as she is in the nest she is in a certain nest-building attitude, a high state of satisfaction, which constitutes the consummatory reaction (Phase II.) of a large cycle. But each time she reaches for a straw, seizes it, and tucks it into the nest, she exhibits thus a little cycle containing a little appetence followed by its own satisfaction.

The time occupied by a cycle varies extremely, from cycles measured in seconds to those that occupy a year or even longer. The relative duration of the phases also is extremely variable. In some cases the appeted situation is attained without delay, and Phase I. thus passes so rapidly as to be overlooked by the observer. In other cases the bird strives hard to overcome great obstacles which stand in the way of the attainment of the appeted stimulus, consequently Phase I. is of long duration. Phase II. may last, in the case of drinking, about one second; in the case of incubation, about three weeks.

It should be stated, too, that the phases are not sharply separated; each passes more or less gradually into the next. Thus, from Phase IV. of one cycle in a series to Phase I. of the succeeding cycle, there is often a gradual rise of appetite; active search for satisfaction does not commence until a certain intensity of appetite is attained. This is what is known in pedagogical literature as "warming up." This gradual rise of the energy of appetite is followed (Phase II.–III., or II.–IV.) by its sudden or gradual discharge. This rise and discharge are named by Ellis ('03), in the case of the sex instinct, "tumescence" and "detumescence." They are important phases in the psychology of art, in which sphere they are named by Hirn ('00) "enhancement" and "relief." The discharge (Phase II.) is also exemplified in "catharsis" in art and in psychiatry.

The cycles in the behavior of birds are fundamentally the same phenomenon as the cycles in human behavior. Human cycles are enriched by an intelligence far surpassing that of doves, but this is a difference of degree only. If the dove's cycles are determined largely by instinct, habit, physiological conditions, and not intelligence, so are some human cycles, as those of sleeping, eating, drinking, sex. F. H. Herrick ('10, 83) emphasizes the fact that a bird may scamp one cycle in order to begin another. Thus, birds may abandon young which are not yet weaned, because their appetence for a new brood has set in. But the same principle works, though not quite so crudely, in human life; as in the case of a mother who grows indifferent or even somewhat hostile toward her older children each time a new child is born. Herrick emphasizes also the fact that when anything disturbs the bird in the progress of a cycle, she very often gives up that cycle and begins a new one. Thus, a cedarbird who has just completed her nest one day finds a man examining it; she forthwith abandons that nest and begins to build another. But, again, the same phenomenon appears in human behavior. A man begins to build a house; when he has progressed far with the building he meets some horrible experience in it which "turns him against" it, and nothing will induce him to proceed with that house; he abandons it and begins to build elsewhere. The cedarbird has had a, to her, horrible experience which has turned her against her nest; that nest has lost its *value* for her; the sight of it now, instead of arousing her appetence, arouses aversion.

C. J. Herrick ('15, p. 61) says that many of these cyclical activities of birds are "simply complex chain reflexes." The reason he gives for this statement is that "each step in the cycle is a necessary antecedent to the next, and if the series is interrupted it is often necessary for the birds to go back to the beginning of the cycle. They cannot make an intelligent adjustment midway of the series." But all this, in some degree, is true of the behavior of human beings toward their mates, their nests, and their young. This has been illustrated in the preceding paragraph, and a few illustrations are here added. As to mates: When the cordial relation between a husband and wife is, by some mischance, broken, the pair may make an "intelligent adjustment" if the difficulty is not too great. But birds also make such adjustments constantly, when the difficulty is not too great. And with human beings, as with birds, the difficulty may be insurmountable; in which case, the husband and wife separate for a week, a month, or a year, after which period of rest (Phase IV.), they can commence a new cycle with Phase I., courtship. As to their nests: The fact of homesickness proves that the behavior of a human being toward his or her home runs in a series which conforms to Herrick's statements. As to behavior toward the young: The inability of human parents to make "an intelligent adjustment midway of the series" is shown by the fact that they cannot arouse the fullest degree of parental behavior toward an adopted child unless they adopt the child in its infancy. These facts do not prove that the human behavior in question consists of mere chain reflexes. Neither do the similar facts as to avian cycles prove that the avian behavior consists of mere chain reflexes.

The birds in their cycles exhibit attention (using this and all the following terms in a strictly behavioristic sense), intelligence, memory, intensely emotional behavior, conflict of tendencies, hesitation, deliberation (of course an elementary sort of deliberation), rise, maintenance, and decline of appetences, behavior conformable to certain laws of valuation. All these forms of behavior function in bringing about the consummatory situations of the cycles. Thus the instinctive behavior of birds, so far from consisting of mere chain-reflexes, and having no relation to "individuality" (Bowen, vide ut supra, p. 97), is in reality very highly integrated, and is the very core of the bird's individuality.

All human behavior runs in cycles which are of the same fundamental character as the cycles of avian behavior. These appear in consciousness as cycles of attention, of feeling, and of valuation.

This description is true not only of our behavior toward objects specifically sought by instinct, such as food, mate, and young, but also of our behavior toward the objects of our highest and most sophisticated impulses. Consider, for example, the course of a music-lover's feelings and attention in the case of a symphony concert. Before the concert, if his internal state is favorable (Phase I.), he is all eagerness, desire, interest. He goes to the concert-hall, chooses a good seat for hearing, and in every way shows appetitive behavior. (Phase II.) The music begins, he pays close attention, and feels satisfaction. (Phase III.) If the concert continues too long, he is surfeited, his pleasure diminishes, he even feels some unpleasantness, and his attention turns away, which is of course a form of aversion. (Phase IV.) When the music at length ceases he feels restfulness, relief, and his attention goes elsewhere. This cycle of the whole concert is overlaid by a complex system of epicycles, each extending through one symphony, one movement, or a smaller division, down to the measure and the beat. This is only one illustration of the fact that the entire behavior of the human being is, like that of the bird, a vast system of cycles and epicycles, the longest cycle extending through life, the shortest ones being measured in seconds. This view helps us to understand the laws of attention; for example, the law that attention cannot be held continuously upon a faint, simple stimulus. For as soon as such a stimulus is brought to maximum clearness, which constitutes the consummatory situation, the appetite for it is quickly discharged and its cycle comes to an end. This familiar fact shows that we, like the birds, are but little able to alter the course of our behavior cycles.

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FURTHER STUDIES ON THE GEOTROPISM OF PARAMECIUM CAUDATUM.[1]

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(With two figures.)

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I. INTRODUCTORY.

As evidence of the existence of proper mechanical conditions in protoplasm as a basis for the "statocyst theory" of geotropism in *Paramecium*, Lyon states: "The animals were strongly centrifuged for several minutes in the hæmatocrit attachment. Microscopic examination showed that certian dark granules originally distributed were now aggregated in one end, usually the anterior. It is thus seen that differences in specific gravity exist in the protoplasm of this animal."[2] In previous experiments in which he attempted to test Lyon's results, the writer "could obtain nothing definite"; and the tentative suggestion was made that "immediately after centrifuging a capillary tube containing *Paramecia*, and in which the latter can not turn around, we may stain them with some dyes and determine the effects of the centrifugal force upon the protoplasmic materials of the organisms."[1]

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[1] From the Physiology Laboratory of the University of Minnesota.

[2] Lyon, E. P., 1905, "On the Theory of Geotropism in *Paramecium*, *Am. Jour. Physiol.*, Vol. 14, p. 430.

While the writer was conducting experiments with a vital stain method in the physiological laboratory of the University of Minnesota, and after he had obtained some valuable results, he learned that McClendon had already published satisfactory results in 1909.[2] The writer found that McClendon's method showed better results than those obtained with vital stains and has, therefore, adopted that method for this investigation, although not able to agree with all of McClendon's interpretations.

This paper attempts to show that *Paramecium caudatum* contains protoplasmic substances of different specific gravities, and subjects to further experimental examination the various phenomena of geotropism in the animal, described by various investigators. The experimental work was done during the academic year of 1913–1914, while the writer was holding a Shevlin Fellowship in the medical school of the University of Minnesota.

II. Material.

A dense culture raised in hay infusion from a single individual was used.

III. Experimental.

1. *The Specific Gravity of Paramecium caudatum.*

As to the difference of the specific gravity of the *Paramecium* between Lyon's 1.048 or 1.049 and the writer's 1.037 or 1.037 ± 0.003, the latter suggested[3] a possible source of error in Lyon's experiments. The writer this time examined the subject more carefully.

He prepared a gum-arabic solution using distilled water, as in the case of his previous experiments. He dialyzed the neutral solution through parchment paper. The specific gravity of the solution so prepared was determined by means of a U-shaped picnometer, as 1.0426.

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[1] Kanda, Sakyo, 1914, "On the Geotropism of *Paramecium* and *Spirostonum*," Biol. Bull., Vol. 26, p. 22.

[2] McClendon, J. F., 1909, "Protozoan Studies," *Jour. Ex. Zool.*, Vol. 6.

[3] Kanda, Sakyo, *loc. cit.*

Then the writer made many small tubes for hæmatocrit attachment, of about 4.7 cm. long and 0.3 cm. in diameter, inside measure. One end of each tube was sealed. One of these tubes was filled with the gum-arabic solution mentioned above and the number of drops which were put in the tube was counted. Now, to the number of the drops of the solution, one drop of water was added. Thus the difference of the specific gravity of the solution before and after the addition of one drop of the latter to the number of drops of the former could be estimated. The writer thus found that the addition of only one small drop of water to the gum-arabic solution of the known specific gravity, that is, 1.0426, lowered the latter to 1.0415. And when another drop of water was added to this, it became about 1.0404. The addition of one drop of water, therefore, lowered the specific gravity of gum-arabic solution about 0.0011.

A pair of tubes was thus prepared with a definite number of drops of the gum-arabic solution of the specific gravity 1.0415. This pair was centrifuged as usual. Then on the top of the solution of one tube, one drop, and on the other, two drops of water containing dense *Paramecia* were added. The tubes were again centrifuged for two minutes with a speed of about 7,300 revolutions per minute. The results were determined by means of a magnifying glass immediately after the centrifugalization. All the procedure was the same as the writer described in his previous paper. Thus the nearest possible density of the animal was obtained:

TABLE I.

I.	Tube 1. Dens. of gum-arab. sol. 1.0404.	All stay at upper part.
	Tube 2. Dens. of gum-arab. sol. 1.0393.	Majority stay near middle.
II.	Tube 1. Dens. of gum-arab. sol. 1.0382.	Many stay at middle.
	Tube 2. Dens. of gum-arab. sol. 1.0371.	A few go to bottom.

This was carefully tried with different cultures and no practical difference was found. The writer, therefore, concludes that the specific gravity of *Paramecium caudatum* is between 1.0382 and 1.0393.

In comparison with the writer's previous results, that is, 1.037 or 1.037 ± 0.003, the difference is not great. In his previous experiments, however, he was not critical enough to test the lowering of the specific gravity of the gum-arabic solution after one or two drops of water containing *Paramecia* were added to the tubes. The writer, therefore, considers that the present result is more correct than the previous one.

A more accurate estimation could possibly be made by having the animals before adding them to the centrifuge tube in gum solution of a density only a little less than that of the animals. The lowering of specific gravity by mixture would thus be lessened. Nevertheless, the writer believes that the assumption of 1.039 as the specific gravity of *Paramecium caudatum* is close enough for biological purposes.

2. *The Effects of Centrifugal Force on the Protoplasm of Paramecium caudatum.*

The specific gravity of the animal being known as about 1.039, a gum-arabic solution of higher specific gravity, that is, about 1.1, was prepared. If *Paramecia* are strongly centrifuged in such a solution, they all should be suspended in the solution. And the heavier end of the animals should, also, be passively thrown away from the axis of the centrifuge. Now the writer put a certain number of drops of the gum-arabic solution in a pair of hæmatocrit tubes already described, and on the top of each, one drop of water containing dense *Paramecia* was added. The solution and the water in the tubes were well mixed by means of a fine glass needle. The tubes were centrifuged for fifteen minutes at a velocity of 108.3 revolutions per second and with radius of 2.5 cm. to 7 cm.

The paramecia with some of the gum-arabic solution, after being centrifuged, were sucked out in a capillary tube and were killed in 1 per cent. chromic acid, being left in the solution for about one minute. Then they were stained by McClendon's method, "in Biondi's methyl green, orange G and acid fuchsin mixture with a little less fuchsin and of about one fourth saturated strength"[1] for about four minutes, dehydrated and mounted.

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[1] McClendon, J. F., *loc. cit.*

The microscopic examination of the preparations showed that the darker and heavier substances and crystal-like materials lay in the extreme anterior end of the animal which was thrown away from the axis of the centrifuge. Next to these came the micro-nucleus and then the macro-nucleus. The chromatin which was stained green, seemed to have been precipitating to the outer nuclear wall, since this part of the nucleus was stained much darker than the inner, as Figs. 1 and 2 have shown. The "plasmosomes" and cell granules were stained orange, but the cilia did not show very distinctly. The writer thus found in confirmation of Lyon's and McClendon's results that *Paramecium* contains protoplasmic materials of differing specific gravities. Figs. 1 and 2 show specimens drawn with the aid of a camera attachment.

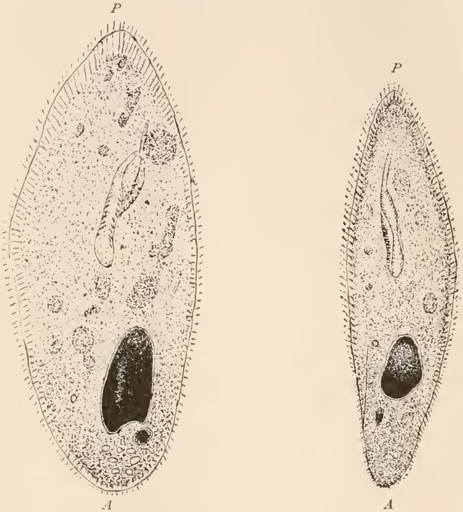

FIG. 1. FIG. 2.

FIGS. 1 and 2. Camera drawings of *Paramecium caudatum*. *A*, anterior end; *P*, posterior end.

That the anterior end of the animal, when centrifuged, was thrown away from the axis of the centrifuge presumably on account of its heavier weight, was originally demonstrated by Lyon. This was the strongest in his experiments to cast aside the "mechanical theory." The writer supported him by furnishing results obtained by improved methods to meet Harper's criticism.[1] Harper insisted that in strong centrifugalization, "the same effect is produced at the outset as by mechanical agitation, *i. e.*, the reaction changes to positive." In other words Harper claims that the head is turned outward by a positive response on the part of the organism, and not passively, as claimed by Lyon. The writer showed in his previous paper that Harper could not be right.

However, McClendon thinks that "the geotropic reaction" of the animal "may be strong enough to turn the anterior end in the opposite direction" toward the axis of the centrifuge against the centrifugal force. This needs a serious examination. If the anterior end of the animal is considered "heavier" than the posterior, as Lyon and Kanda hold, it is hardly conceivable that such a small animal as *Paramecium* could orient itself with the heavier end toward the axis of the centrifuge against the force McClendon used. On the other hand, if the posterior end is regarded "heavier" than the anterior, as Verworn[2] and Harper hold, one meets the same difficulty to conceive how the animal can orient itself with the "lighter" anterior end away from the axis of the centrifuge against the much greater force used by Lyon and the writer. McClendon admits that "this is usually the initial orientation," but seems to think the animals may occasionally turn around later by an active reaction. The writer must confess that he cannot conceive its possibility. He found one individual among several dozen examined which was thrown with the posterior end away from the axis of the centrifuge. He considers, however, that this was a mere accident, the animal being oriented in that direction at the beginning of centrifugalization and forced into the capillary before passive turning could occur. McClendon's case was also probably exceptional and moreover had been centrifuged twenty-four hours, during which admitted changes in specific gravity had occurred, due to loss of water. If this loss were chiefly from the posterior part, the changed orientation would be explained as a passive instead of an active process. The writer, therefore, holds still that these animals, when strongly centrifuged, are *passively* thrown with their anterior ends away from the axis of the centrifuge. Therefore, the anterior end is heavier. The negatively geotropic orientation in the normal animals is, therefore, an active process and the mechanical theory is not correct.

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[1] Harper, E. H., 1911, "The Geotropism of *Paramecium*," *Jour. Morph.*, Vol. 22, p. 998.

[2] Verworn, Max, 1889, "Psycho-Physiol. Protisten-Studien," Jena: Gustav Fischer, p. 121.

McClendon "found the time elapsing before the return of the negatively geotropic reaction to roughly correspond to the time required for the return of the nuclei to their normal position." The writer also found that this was the case. According to McClendon, "this might indicate that nuclei in normal position acted as statoliths." It is hard to decide for or against this for at the time of "the return of the nuclei to their normal position," other heavy substances, "phosphate crystals," for instance, which were precipitated in the extreme anterior end of the animal seem, also, to distribute in their original position. In other words, about at the time of return of the nuclei to their normal position, all the protoplasmic substances of the animal, which were disturbed by the centrifugal force from their original distribution, recover from the disturbance. The result is that the animal resumes the negatively geotropic reaction. Such consideration may suggest that the whole organism in normal conditions acts as a "statecyst," and all the heavy substances as "statoliths," not merely the nuclei. Here lies the significance of Lyon's conception that "for internal stimulation the relation of the parts of the cell to each other must be changed in some way by gravity. Stresses or pulls which occur when the organism is in one position with respect to the vertical, must be changed in another position." For the present we cannot decide in favor of any particular organ or constituent of the cell as the basis of the reaction.

3. Does temperature reverse the negative geotropism of *Paramecium caudatum?*

From his observations on the effects of raising and lowering temperature on the geotropism of this animal, Sosnowski concludes: "Es hat sich dabei herausgestellt, dass durch Einwirkung höher Temperaturen . . . bei vielen Culturen vorübergehend positiver Geotropismus hervorgerufen werden kann." And "durch die Temperaturerniederingung (bis + 2°) konnte ich auch bei den sehr dazu geeigneten Aquarien keinen positiven Geotropismus hervorufen."[1] On the contrary, Moore[2] states: "Several tubes were left for three hours in a thermostat at a temperature of 26°–28° C. The *Paramecia* collected at the top in dense clusters." And "tube *B* was placed in a larger tube filled with water and surrounded by a mixture of ice and salt, the temperature being kept as nearly as possible at 1° C. In ten minutes the *Paramecia* in tube *B* were massed at the bottom, and two hours later were still massed there."

Sosnowski and Moore, however, gave no consideration to the phenomena of the convection currents which would invariably occur, when water was cooled or heated. That water currents would produce effects on the geotropism of the animal was mentioned by the writer in his previous paper. The animals are rheotropic. Moreover, the animals being so small cannot resist any strong current. Consequently they would be passively carried by the current, when it was strong. There was, therefore, needed a special device to minimize these effects as much as possible.

Dewar vacuum tubes served to some extent for this purpose. The tubes were 3.2 cm. in diameter and 27 cm. long inside measure. Three tubes, one for control and the others for experiments, cold and warm, were used for one series of experiments. It was noted that warm water of 30° or 35° C., which was placed in one of the tubes, with a rubber stopper, cooled off about 2° C. after one hour, and water of 1° to 4° C. in the other became warmer 2° C. in about half an hour. Temperature of the control, that was room temperature, was about 20° C. and fairly constant. The dense culture of animals that were thoroughly washed was cooled or warmed as desired. And a few drops of the culture so prepared were transferred by means of a pipette into the Dewar tube with special care to make the least current. The tube was closed with a rubber stopper. The results based on many experiments are given in Table I.

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[1] Sosnowski, J., 1899, "Untersuchungen über die Veränderungen der Geotropismus bei *Paramecium aurelia*," *Bull. Intern. d. l'Acad. d. Sci. d. Cracovie*, S. 134.

[2] Moore, Anne, 1903, "Some Facts concerning the Geotropic Gathering of *Paramecium*," *Am. Jour. Physiol.*, Vol. 9, pp. 239 and 240.

TABLE I.

EFFECTS OF TEMPERATURE (?).

Time.	Cold.	Control.	Warm.
	1°–2° C	20° C.	20°–30° C.
Immediately after.	Very sluggish and hardly active.	Majority swim downward.	Abnormally active and majority swim down.
5–10 m. later.....	Not very active and scattered all over.	Many at the lower part.	Many at the lower part.
20–30 m. later....	A little more at the lower part and becoming somewhat active. The temp. about 4° C.	Many at the upper part.	A little more at the lower part. The temp. about 29° C.

Cooling water to 3°, 4°, or 5° C. and warming to 35° C. were also tried. At a temperature 5° C., the animals were somewhat active, and showed a tendency to swim downward. Another series of experiments was also carried out in an incubator and in an ice box. Each experiment lasted three days. The temperature in the incubator ranged from 30° C. to 34° C. In one series of the experiments, the animals so treated in the incubator were "positively geotropic"? That is to say, a majority of the animals always staid at the bottom of the tube, as Sosnowski observed. But in others, the results were very irregular and variable. Experiments in the ice box were not satisfactory, because it was very hard to keep the temperature constantly low.

The writer found that the animals always swam downward, whenever transferred, no matter how carefully it was done. This occurred invariably even in water of the same temperature as the culture, when the animals were transferred into it. It seems to the writer, therefore, to be possible that this reaction was mistaken as reversibility of geotropism of the animals by temperature. That the animals swim downward, *i. e.*, their negative geotropism is reversed, whenever transferred, would suggest that mechanical agitation of transferring may be responsible for it. Sosnowski also suggested this factor in one case, "die Temperaturerhöhung und Erschütterung sich zu summieren scheinen" The reversibility of the negative geotropism of the animals by mechanical "shock" or agitation is a well-known fact. The water current may also help it to some extent.

From these results the writer concludes that so-called reversibility of the negative geotropism of *Paramecium* by temperature is extremely doubtful.. Especially in such cold water as near 1° C., the animal is almost, if not thoroughly anesthetized. In consequence, it sinks by its weight, though Moore thinks it is "reversed."

4. Do chemicals reverse the negative geotropism of *Paramecium caudatum?*

According to Sosnowski, "durch Zusatz geringerer Mengen von Säuren oder Alkalien (1 bis 3 c.c. Salzsäure oder Natronlauge von 0.5 per cent. auf 20 c.c. Culturflüssigkeit) kann man verubergehenden positiven Geotropismus hervorufen." Moore also states that "in $n/16$ NaCl, they went to the bottom almost immediately, but shortly after died." In a solution of calcium chloride isosmotic with $n/32$, "they went immediately to the bottom."

The writer attempted to test these statements of Sosnowski and Moore. First of all, he thoroughly washed the animals in boiled then cooled tap water. Chemical solutions from the strongest to the weakest were carefully prepared using boiled tap water. A few drops of culture containing dense *Paramecia* were transferred with special care to avoid making water currents as much as possible. The results given in Table II. are to be considered as the best parts of many series of experiments that were quite extensively carried out.

As the table shows, the majority of the animals always swam downward, *i. e.*, were positively geotropic, whenever transferred into any solution as well as in the control, *i. e.*, transfer into culture water. Among the substances used, perhaps, $CaCl_2$ solution "affected" the animals most. In this respect, Moore's observation seems to be confirmed, but it needs further consideration. In any solution, the animals that swam to the bottom did not stay there very long. They resumed their negative geotropism within one hour. Sosnowski already observed the same and stated that "wenn sich darauf die Tiere wieder an der Oberfläche sammeln, kann man durch Zusatz einer neunen Quantitat Säure resp Alkali wieder die Ansammlung am Boden versanlassen." This statement is true. But it should be remembered that the same is also true when some tap water is added. It seems to the writer that this means that the so-called reversible effect of chemicals on the negative geotropism of *Paramecium* is not specific but general. Moreover, the animals, whenever transferred to another receptacle without changing the solution, swim downward, and reversed animals do not stay long at the bottom.

TABLE II.

EFFECTS OF CHEMICALS.

	Room Temp. 20° C. Time.					
	Immediately after Treatment.	5 Minutes after Treatment.	10 Minutes after Treatment.	20 Minutes after Treatment.	30 Minutes after Treatment.	60 Minutes after Treatment.
Control........	90% or more swim downward.	About 85% at bottom.	Number at bottom decreasing.	Scattered all over.	About 70% at upper part.	About 80% at top.
0.01 m. or 0.0133 m. $CaCl_2$......	Abnormally active and 95% or more swim downward.	About 95% at bottom.	About 80% at bottom.	About 60% at lower part.	60% or more at upper part.	About 85% at top.
0.01 m. or 0.0133 m. Na_2CO_3 or $NaHCO_3$......	About 95% swim downward.	85% or more at bottom.	Scattered all over.	About 70% near top.	80% or more at top.	About same.
.05% HCl[1].....	90% or more swim downward.	70% or more at bottom.	Scattered all over.	About 60% at upper part.	About 70% near top.	About same.

There is, therefore, reason to believe that this is the same phenomenon that we met in the temperature experiments. The writer concludes, therefore, that mechanical "shock" or agitation of transferring, or chemical "shock," is chiefly responsible for so-called reversible effect of chemicals on the negative geotropism of *Paramecium* but not chemicals themselves.

Besides the chemicals mentioned above, sodium chloride, magnesium chloride, cane sugar, ethyl alcohol and chloroform in appropriate strength were tried but no better results were obtained.

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[1] This was adopted as approximately Sosnowski's strength.

IV. Summary and Conclusions.

1. The specific gravity of living *Paramecium caudatum* is between 1.0382 and 1.0393.

2. *Paramecium caudatum* contains protoplasmic materials of different gravities.

3. *Paramecium caudatum*, when strongly centrifuged, assumes a position with its anterior end directed away from the axis of the centrifuge. In so doing, it must be passively thrown by the centrifugal force, because the centrifugal force used was altogether too strong to be resisted by the animal. For this reason, its anterior end must be heavier than its posterior end. The negatively geotropic orientation, therefore, is an active process and the mechanical theory is not correct.

4. The whole organism seems to be a "statocyst," since the recovery of negative geotropism after centrifugalizing is synchronous with the reëstablishment of normal relations of substances in the cell.

5. So-called reversibility of the negative geotropism of *Paramecium caudatum* by temperature and by chemicals is extremely doubtful. Mechanical "shock" or agitation is chiefly responsible for the reversal one sees in such cases.

In conclusion the writer wishes to acknowledge his indebtedness to Professor E. P. Lyon, under whose direction this work was completed, and who gave valuable suggestions and criticism of the work and manuscript. His thanks are due also to Dr. J. F. McClendon, who gave his generous help and suggestions during the experiments.

NOTE ON THE GEOTROPISM OF PARAMECIUM.

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Harper's[1] criticism of my experiment demonstrating, as it seemed to me, that the anterior end of paramecium is the heavier and consequently that the negative geotropism of this animal is an active process was founded upon the assumption that when one begins to centrifuge the animals their geotropism is reversed and becomes positive. Thus the animals start, according to Harper, to move in the direction of the accelerating force and consequently away from the axis of the centrifuge. Harper's experiment by no means proves his contention and it always seemed to me, as Kanda brings out in the foregoing paper, that the quickness with which a high centrifugal force is developed in the centrifuge would prevent the animals from orienting themselves before they were thrown into the capillary tube where turning is impossible. However, this suggestion might not seem proof to some people; and I have, therefore, performed the following experiment:

A culture of paramecium was cooled almost to 0° C. At this temperature they move very sluggishly and show no distinct orientation to gravity. They gather at the bottom of a test tube due, I am sure to the fact that their specific gravity is greater than water and that they make no movement sufficient to keep themselves suspended. The centrifuge was prepared with capillaries such as Kanda and I have used but each one immersed in a larger tube containing ice. A drop of the cold paramecium culture was quickly transferred to the cold capillary tube and centrifuged. The animals were always precipitated with their anterior ends away from the axis. I am quite certain that the animals at this temperature make no reaction to gravity or agitation and that the proof may be considered definite that the head end of the animal is the heavier and that the ordinary geotropic orientation is an active process.

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[1] For the literature see the preceding article by Kanda.

THE MICROVIVISECTION METHOD.

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The dissection of living cells has hitherto proved inadequate for a study of the physical properties of protoplasm and of its structural components. This is owing largely to a lack of means for the manipulation of dissecting instruments under a sufficiently high magnification of the microscope.

Before and since Barber first devised his mechanical pipette-holder for the isolation of bacteria, various ingenious methods for the dissection of microscopic objects have been described. They all, however, fall short of Barber's method, because, with them, only comparatively low powers can be used, since the dissecting needles must operate *between* the objective and the tissue to be dissected. On the other hand, by using needles instead of micro-pipettes, Barber's instrument has been converted into an excellent microdissection apparatus. The needles operate in a shallow hanging drop containing the cells to be dissected which are pressed against the *undersurface of a coverslip* in a moist chamber. There being no obstacle above the coverslip, oil immersion lenses may be used for observation.

In an article appearing as long ago as 1859, Dr. H. D. Schmidt, of Philadelphia, describes in detail an excellent "microscopic dissector" consisting of a base to be fastened on the stage of a microscope with a number of clamps to hold instruments, each clamp possessing three movements controlled by screws. A lever fastened in one of the clamps holds the tissue in place. Fine scissors, knives or steel needles are fastened in the other clamps. By turning the various screws, the instruments can be brought into place and be operated with remarkable accuracy. Dr. Schmidt worked with the tissue, the instruments and the lower lens of the objective immersed in water or diluted alcohol. The full results of his investigations with this instrument were not published until 1869, owing to interruptions due to the Civil

War, during which his drawings and manuscript were burned in a conflagration in Washington. In his later paper he has two illustrations showing a liver cell being torn in two by needles.

In 1887 Chabry described an apparatus in which a glass needle was held in a sheath in such a way as to allow the needle to be pushed to any prescribed distance into the bore of a glass capillary tube. The bore of the capillary was made just large enough to admit a single ovum and hold it in place. By proper adjustments, the needle could be pushed to any depth in the ovum. The apparatus was placed on the stage of a microscope and Chabry was able to injure locally a *Strongylocentrotus* ovum while under observation. Chabry's instrument has been used for experimental embryological work and is described in detail in Ehrlich's "Enzyklopädie für microskopischen Technik," 1910.

In 1907 J. F. McClendon first described his "mechanical finger," which consisted of an ordinary Spencer mechanical stage with an additional screw to allow of movements in three different directions and a clamp to hold a needle or pipette. With this apparatus, McClendon was able to suck the nucleus out of a *Chætopterus* egg. In 1909 he described an improved form with which he dissected certain Protozoa.

In 1912 an apparatus was described by Tschachotin. It consists of a clamp attached to the side of the objective of a microscope. In the clamp is fastened a needle which curves so as to project under the objective and is so adjusted that the tip lies in the focus of the objective. The needle point is lowered into the object to be dissected simply by bringing the object into view under the microscope and lateral tears are made on moving the object with the slide by means of the mechanical stage.

In 1904 Barber first described his method of using a hanging-drop in a moist chamber for isolating microörganisms. A fuller account appeared in 1907 in which he first describes a mechanism for holding micropipettes and where he mentions Montrose T. Burrows as having assisted him much in its design. Elaborations of his instrument appeared in his papers of 1908 and 1911. In 1911 he suggested the possibility of the use of his instrument for cell dissection and for investigation on fertilization and heredity problems. His paper of 1914 is a detailed and precise description of his technique and is of the utmost value for the prospective worker in micro-dissection. As he developed his method principally for the use of pipettes and also because his latest paper appeared in a publication not readily obtainable, I have endeavored to give here a description of the method with modifications and new developments in the application of the method to microvivisection.[1]

A simple form of Barber's apparatus is shown in Fig. 1. The moist chamber, which is open at one end and with sides from 8 to

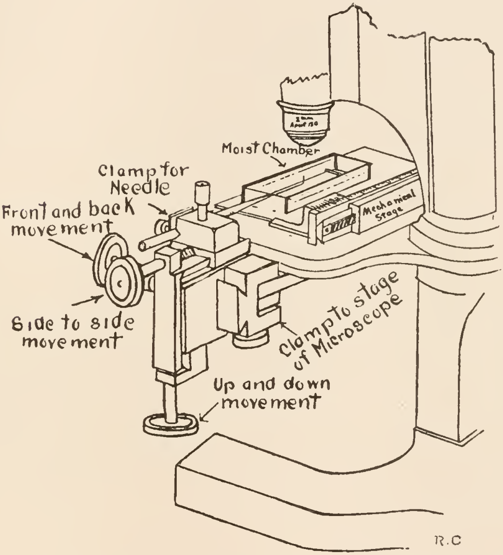
Moist Chamber
Clamp for Needle
Front and back movement
Side to side movement
Mechanical Stage
Clamp to stage of Microscope
Up and down movement
R.C

Fig. 1. Barber's single three-movement pipette holder, glass needle and moist chamber arranged to illustrate method of dissecting cells in a hanging drop under the highest magnification of the microscope. (Substage of miscroscope omitted in drawing.)

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[1] In 1912, G. L. Kite first applied Barber's apparatus to microdissection, which quickly resulted in the publication of several papers. Kite was to have prepared an article on the method when his health unfortunately broke down. Publications on microdissection have appeared as follows: 1912, Kite (on the fertilization membrane) in *Science*, N. S., XXXVI.; Kite and Chambers (on chromosomes and nucleus) in *Science*, N. S., XXXVI. 1913, Kite (on permeability of cells) in Biol.

12 mm. high, is placed on the microscope so that it may be moved about with the mechanical stage. The chamber is roofed over with a carefully cleaned coverslip, on the under surface of which, the specimen is mounted in a shallow hanging drop of a physiologically indifferent fluid. The dissecting needle is made by drawing out one end of a piece of glass tubing and bending it at right angles two or three millimeters from the pointed tip. The needle-holder, a mechanism allowing of three movements, is clamped to one side of the microscope stage and the needle is adjusted so that it may project into the moist chamber with its tip pointing up into the hanging drop. By proper adjustment the cell to be dissected and the point of the needle are brought into the same focal field. The three movements of the needle, permitted by the needle-holder, and the two movements of the moist chamber, by means of the mechanical stage, give the experimenter ample opportunity to carry on dissection under the highest magnification of the microscope.

THE INSTRUMENT.

The holder accommodated to carry only one pipette or needle is illustrated in Fig. 1. It is useful in many ways and is comparatively inexpensive. Its disadvantage lies in the fact that the resistance of the cell-substance to the needle may overcome the inertia of the cell, so that, instead of tearing through the cell, the needle simply drags it about. However, by careful manipulation and by the possible use of a viscous dissection-medium much can be done. Many marine ova and germ cells in general are very soft in consistency and, if the drop in which they are immersed be shallow enough, the surface tension of the drop flattens them against the coverslip and is sufficient to hold the cell. The single holder can also be successfully used for injecting material into or extracting material out of a cell. In addition to the movement of the needle, manipulation is greatly aided by the

BULL., XXV., and (on protoplasm) in *Amer. Jour. Physiol.*, XXXII., 1914, Chambers (on the nucleus) in *Science*, XL.; Kite (structural transformations of blood cells) in *Jour. Inf. Dis.*, XV. 1915, Chambers (on the germ cell) in *Science*, XLI.; and (on protoplasm) in *Lancet-Clinic*, March 27, Cincinnati; Kite (on cell permeability) in *Amer. Jour. Physiol.*, XXXVII. 1917, Chambers (on protoplasm) in *Amer. Jour. Physiol.*, XLIII.; and (on the cell aster) in *Jour. Exp. Zoöl.*, XXIII.

use of the mechanical stage by means of which the moist chamber may be moved in two directions.

The double holder, formerly manufactured by the Fowler shops of the University of Texas, is twice as expensive as the single holder. It possesses, however, the advantage that two needles or pipettes may be used simultaneously. Each needle can be moved independently of the other and either needle may be replaced by a pipette. The manufacture of the holder has recently been duplicated by the mechanician of Wesleyan University under the direction of Dr. H. B. Goodrich with modifications of his and my suggestion. In setting up the apparatus, Dr. Goodrich introduced the useful innovation of having the shelf to which the instrument is clamped on a stand independent of the microscope. The two stands are then clamped together on a common base. The operator can thus shift the position of the instrument placing it at will in front of or on either side of the microscope.

Fig. 2 illustrates the double holder as I have it set up. The attachment of the instrument to the front of the microscope stage facilitates manipulation of the screws with both hands. In the illustration, a Spencer Lens Company lamp replaces the substage mirror. As the heat of the lamp, however, produces undue evaporation and subsequent condensation in the moist chamber, it is preferable to use the mirror. This necessitates raising the microscope-stand on a block of wood so that the mirror can be lowered below the microscope base so as to receive light unobstructed by the lower screws of the dissecting instrument.

The needle-carrier with its groove (Fig. 2, *a*), in which the arm of the needle lies, is twice as long as in Barber's original instrument. This extra length assists one in giving a straight even movement to the needle when it is being pushed into the moist chamber. After the needle has been thus adjusted by hand approximate to its proper position, the plate *b* is pushed over the arm of the needle and the screw *c* tightened to clamp the needle. Further adjustment of the needle is carried out by the screws *e*, *f*, *g* and *h* under the low and high powers of the microscope.

In preparing the shelf on which the instrument is to be clamped (Fig. 2, *j*) care must be taken that it be low enough to bring the top of the carrier flush with the upper surface of the microscope

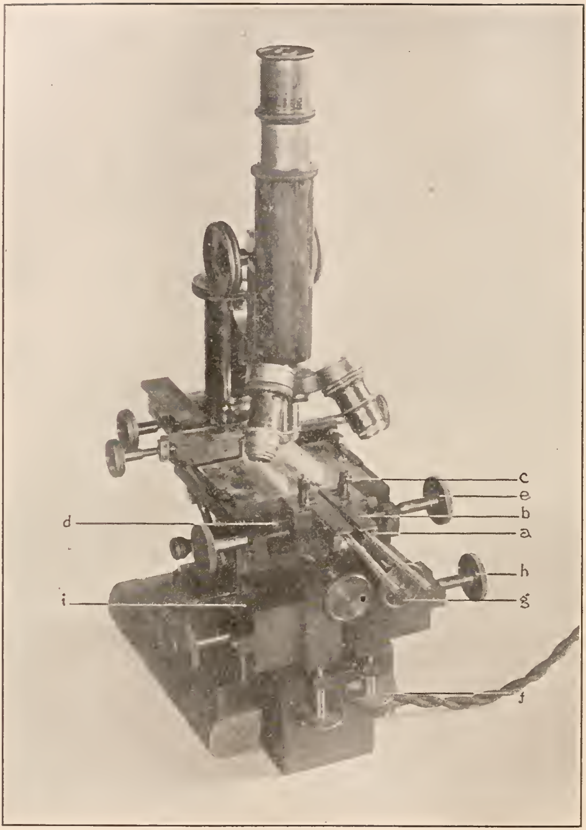

FIG. 2. Double pipette holder set up for microdissection. *a*, Carrier with groove to hold needle. *b*, brass plate which slips sideways over the groove in which needle lies. (The undersurface of the plate is covered with blotting paper to give it resiliency where it comes into contact with the glass needle.) *c*, set screw to clamp plate on carrier. *d*, screw which, on loosening, allows carrier to be set at any angle. *e–h*, screws to move the needle (*e*—side to side, *f*—up and down, *g*—in and out, *h*—side to side for both carriers together). *j*, shelf to which instrument is clamped.

stage. This is in order to enable one to use a moist chamber of the minimum height (see below).

An important factor in the manufacture of the microdissection instrument is that the two horizontal movements run true and keep the needle point always in the same plane. Otherwise, at a critical moment in the dissection, a reversal of a horizontal screw may either suddenly lower the needle-point out of focus or jam it against the coverslip and break it. Unfortunately, the last model put out by the University of Kansas is deficient in this respect. The existence of lost motion in the screws can be taken care of in the manipulation but if the needle tip does not run true in a horizontal plane the peace of mind of the operator may be sorely tried!

THE MOIST CHAMBER.

The moist chamber (Fig. 3) is a glass slide to which are cemented strips of glass in such a way as to form a box open at the top and at one end. For cement Canada balsam may be used.

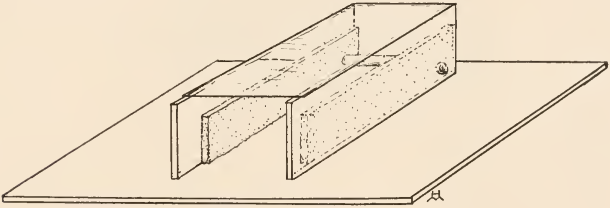

FIG. 3. Moist chamber for microdissection.

A shallow trough, to hold water, is made with a narrow glass strip set across the floor of the chamber 8 or 9 mm. from the closed end. This and other small strips set outside the chamber serve to reinforce the walls. Care must be taken that the upper edges of the chamber be even and level when placed on the stage of the microscope. A carefully grease-free cleaned coverslip with a hanging drop containing the objects to be dissected forms the roof of the chamber. The roof is sealed on with vaseline smeared along the upper edges of the walls of the chamber.

The height of the chamber is conditioned by the focal distance of the condensor used and by the minimum working room that one must have in which to manipulate the needles. For routine work it may be well to have a chamber of fairly large dimensions disregarding the condensor as it is surprising how much illumination a condensor will give beyond its focal distance. However, for critical work, it is imperative to have the height of the chamber such that the objects on the undersurface of the coverslip be close to the focus of the condensor. My condensor has a working focal distance of almost 9 mm. and the chambers I use are 45 mm. long, 22 mm. wide and 9 mm. high.

The moist chamber is lined on the sides with blotting paper leading from the trough to carry the water along the length of the chamber so as to furnish a large moist surface. The liquid in the trough and the blotting paper should be isosmotic with the liquid used for the hanging drop so that evaporation be equalized and the hanging drop not become diluted.

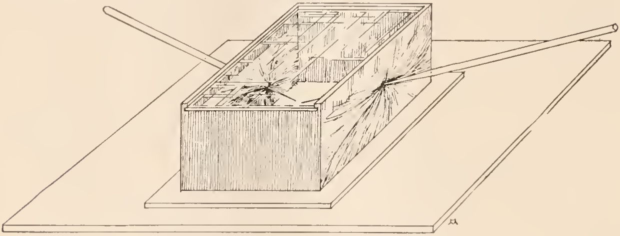

FIG. 4. Moist chamber for preliminary teasing of tissue.

For the dissection of the cellular elements of somatic tissues, it is necessary to do some preliminary teasing under an ordinary dissecting microscope. This should be done in a moist chamber of some kind. The tissue is teased on a coverslip which is then inverted and placed on the microdissection moist chamber.

A handy moist chamber (Fig. 4) can be made out of a shallow box with dimensions approximately 7 × 5 × 3 cm., with a top and bottom of glass and the two longer sides of thin rubber sheeting. The coverslip with the tissue to be teased is placed within the box and the teasing is carried out with needles projecting through the rubber sheeting. For the tissue of warm-blooded animals both the preliminary teasing and the microdissection should, of course, be carried on in warm boxes.

Tissue cultures are grown on square coverslips. The slips are then placed on the moist chamber for microdissection, the parts of the chamber not roofed over being previously covered with slips of glass.

THE NEEDLES.

These are made from glass tubing with an outside diameter of about 4–6 mm. and a lumen of 3–5 mm. The technic is readily mastered with soft glass tubing but needles made of hard glass are more durable.

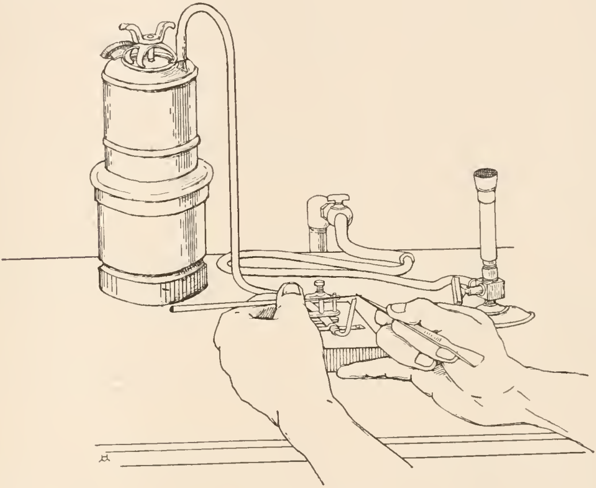

FIG. 5. Method of making the capillary pipettes and microdissection needles.

The needle points are made over a minute flame. A serviceable micro-burner with an acetylene generator is shown in Fig. 5. The microburner is made out of a piece of hard glass tubing bent almost at right angles and with one end closed except for the smallest possible aperture that will retain a flame. This is done by heating the end and pinching it with a pair of forceps. When set up the tip of the burner should be at a height of about 5–6 cm. above the surface of the table. The size of the flame is regulated by the screw pinch-cock set on the rubber tube connecting the burner with the gas generator.

As a source for the flame acetylene is to be preferred to ordinary illuminating gas because with the former a narrower flame can be obtained without undue clogging. The acetylene generator in the figure is part of an old bicycle lamp. A more convenient way is to have the acetylene in a small compression tank which can be recharged at a very small cost. If, however, ordinary gas is to be used, one may much improve the gas by passing it through alcohol or benzene.

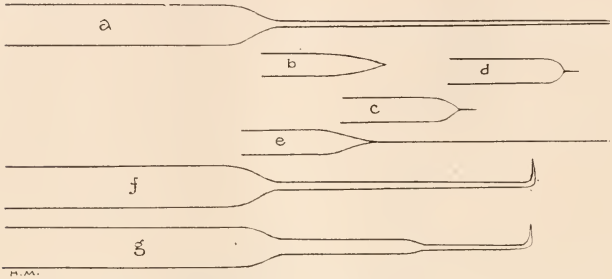

FIG. 6. Stages in making needles. *a*, glass tube with one end drawn out into a capillary. *b*, good tapering point. *c* and *d*, serviceable points. *e*, tip drawn out into a hair. *f*, completed microdissection needle. *g*, needle made on a relatively stout shank.

The method of procedure in making the glass needle is as follows:

1. In an ordinary Bunsen burner (or a Mecca burner if hard glass be used) draw out one end of a piece of glass tubing into a straight capillary about 0.3–0.5 mm. in outside diameter (Fig. 6, *a*). Prepare a supply of such pieces with the capillary ends at least 6 cm. long.

2. Lower the flame of the microburner to the smallest flame that will remain lighted. The microburner should be protected from draughts of air and in semi-darkness so that the flame may show up to best advantage.

3. Hold the shank of the glass tube in the left hand and, with a pair of fine forceps held in the right, grasp the capillary at a point about 5 cm. from the shank. Bring the portion of the capillary next the forceps over the flame and at right angles to it at a point just over the flame (Fig. 5). Pull gently with the

forceps and, when the glass begins to soften, lift it slowly from the flame and pull with the forceps slightly more than at first, but not too strongly. The hands should remain on the table during the process and the pulling and lifting done by turning them slightly outward. The capillary will separate with a slight tug—a feeling much like that experienced when a taut thread, held in the fingers, is parted in a small flame. If the point is properly made, it will appear as shown in Fig. 6, *b*, *c* and *d*. This capillary has sufficient rigidity and it comes to a very fine point. The tip is closed, but the lumen extends almost to the end. It is evident that everything depends upon the amount of heat used and the timing of the pull, and that these must vary slightly with the height of the flame and the diameter of the capillary. With a little experience, one can usually tell when a proper point is made by the peculiar feeling described above. If too little heat is used and the pull made too suddenly, the capillary may part with a snap and the tip be found broken off short often forming a serviceable pipette. If too much heat is used, the capillary is apt to be drawn into a long hair (Fig. 6, *e*).

4. After a suitable point is made, the end of the capillary is turned up at right angles (Fig. 6, *f*). This is done by holding the point of the capillary just back of the point above the small flame and pushing up the end with the tip of the forceps or with a needle. Not more than 5 mm. should be turned if it is to be used in a chamber not over 8 mm. in height. If a good point is made, *e. g.*, Fig. 6, *b*, it is not necessary to turn the end at exactly right angles. A pipette is made by jamming the tip of a needle against the cover glass in the moist chamber to break off the point.

The forceps used in the making of the needles should be of a size that can be firmly grasped in the hand. The fine tips should be accurately set and almost parallel one another for a few millimeters. In order to give the tips a good, grasping, resilient surface I dip them, before using, in a small vial of Sandarac varnish which may be obtained from a dentists' supply dealer. As the varnish burns if brought too close to the flame, it is well to use a second forceps or a needle for bending the glass capillary.

Fine points are not readily made from capillaries of a diameter greater than .05 mm. Therefore, if the dissection requires a comparatively stiff shank for the dissecting needle, start with a glass tube possessing a thick capillary and draw out the tip into a thinner capillary. This thinner capillary may then be used for making the needle point (Fig. 6, *g*).

The facility in making the needle-points over the microburner increases, of course, with practice. In my case, I make at times twenty or thirty within an hour, at other times none, or only one or two at the most. A supply may be kept on hand on a stand made of two horizontal layers of wire netting, the meshes of which admit the base of the needles. It is well to mark off the stand into compartments to receive the various types of needles that are made.

Another good method for making the needle points is that of Chabry ('87). The tip of a capillary pipette is brought into contact with a heated mass of glass (or any incandescent body to which glass will adhere) and suddenly drawn away. For an incandescent body Chabry used the blade of a platinum knife of a surgical instrument for thermocautery. The glass capillary was held by the operator in a groove on a stand a few centimeters from the platinum blade, which was fastened so as to be immovable. An assistant controlled the heating of the platinum blade. As soon as it heated to a dull glow, the operator slid the capillary on its groove until the tip touched the glowing metal when it was instantly slid back. The sliding of the capillary in a stationary groove insures a tapering to a point in a straight line with the long axis of the capillary. In his paper Chabry discusses this method in some detail, pointing out ways of avoiding difficulties in the technique ('87, pp. 175–178). Chabry also suggested the use of insect mouth parts, annelidan bristles and the spicules of sponges for the tips of microdissection needles. Barber suggested fine-pointed needle crystals, or the sharp, stiff hairs taken from the body of a house fly. A pipette with a fairly large opening is made and the hair or crystal is drawn partially into it. The fine point projecting from the tip of the pipette is then used as a probe or dissecting needle. Such needles could be used only on very delicate soft objects.

Needle points may also be made by grinding one end of a fine wire. To produce chemical effects in a cell, McClendon ('09) used a copper wire ground to a point and further sharpened by erosion in acid. The chemical injury produced by metal needles limit their use for the dissection of living cells.

For experiments in cutting cells, one must take into account the fact that many cells will lose their more or less fluid contents if a gap be torn in their surface. If, however, the side of a fine needle can be brought to press the cell against the coverslip, a constriction will result which may cut the cell into two intact pieces. For cutting soft-bodied ova and protozoa this can be done with a form of needle depicted in Fig. 7. The needle tip is bent back rather than forward to lessen the chances of breaking the tip when adjusting the needle in the moist chamber and also when the needle needs to be cleaned (see below). The tip should not point directly back but slant off to one side (see Fig. 7, *b*), so that the shank of the capillary will not be in the way of the light coming up from below. The angle at which the needle is bent must be ascertained by successive trials, for, if it be bent too near to a horizontal plane, the tapering tip will bend away from the cell instead of cutting into it. If the angle be too far from the horizontal plane, the cell will slip along the needle and escape. When the proper angle is obtained, one can cut *Arbacia* and *Asterias* ova into pieces of almost any required size with considerable ease. A clean cut through an unfertilized sea-urchin egg or a fertilized egg, before the fertilization membrane has become too tough, can be best made by bringing the egg between the horizontal arm of the needle and the lower surface of the hanging drop (Fig. 8). On lowering the needle out of the drop,

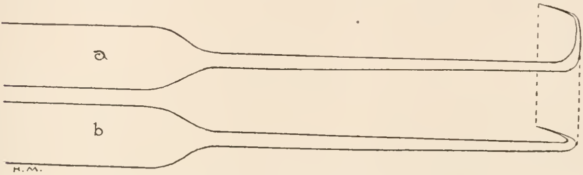

FIG. 7. Needle with tip curved back to almost an horizontal plane. *a*, lateral view. *b*, view from above.

the arm of the needle passes through the egg cutting it cleanly in two.

A good needle, when once made and proven to be resilient and strong, may be used repeatedly. Mucinous material which soon covers its tip so as to render it useless can be got rid of by immersing the needle successively in a strong acid and a strong alkali.

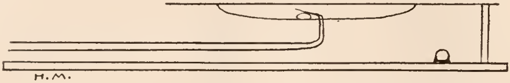

FIG. 8. Side view of moist chamber to show method of cutting an egg cell in two with needle shown in Fig. 7.

CELL INJECTION.

Barber, in his two papers ('11 and '14) gives a full description of his admirable mercury method for injection. With it one can inject microscopically minute doses of a solution into any part of a cell. The advantage of the pressure produced by the mercury over that by air lies in the slow driving force of expanding mercury which can be almost instantly arrested when the dose injected is considered sufficient. On the other hand, if air pressure be used, the air in the injection tube will compress enormously as the resistance of the bore at the tip of the pipette is too great to overcome. If the injection does occur it may go with a rush usually followed by a volume of air which destroys the cell being experimented upon. Notwithstanding this, it is surprising what delicate work may be done simply with air pressure. An example of this is the extraction of the nucleus from a mature *Arbacia* egg and its subsequent injection into another egg. For the injection of substances, however, where the amount must be strictly controlled, the mercury method is essential.

The extraction of material out of a cell is comparatively easy to carry out, as the capillary attraction in the lumen of a micropipette (the inside diameter of which may be anywhere from 2 micra up) is quite sufficient for the purpose. For injection purposes the difficulty lies in securing the force to overcome this capillary attraction.

SOURCE OF LIGHT AND LENSES.

A most serviceable light for microscopic work is the 100-watt horseshoe filament, Edison, Mazda, nitrogen lamp with the blue daylight glass. This lamp, when set in a frosted milk-white glass globe, gives a white light very restful to the eye.

In selecting lenses care should be taken to procure those possessing the maximum focal length together with the greatest numerical aperture. For this reason the Zeiss apochromatic 4 mm. dry lens with a N. A. 0.95 and the 3 mm. oil immersion lens with N. A. 1.40 are excellent for microdissection or for the study of living tissue in general. It is to be remembered that the equivalent focus of a lens does not correspond with the working focal distance.

A good substage condensor with a working focal distance of 8–9 mm. can be made by removing the top lens of the Leitz triple-lens, centering condensor. The beam of light issuing from it completely fills the back lens of a 4 mm. objective with N. A. 0.65 and more than half of a 1/12 oil immersion objective with a N. A. 1.30. This gives enough definition for such detailed cytological work as the study of the chromomeres in grasshopper germ-cells and of the types of protoplasmic granules in marine ova. One may obtain very good condensors with a long working focal distance from those used in projection apparatus where a cooling trough is inserted between the condensor and the microscope stage.

SETTING UP THE INSTRUMENT.

The instrument is first clamped to the microscope and the moist chamber, with the edges of its walls smeared with vaseline, placed in the mechanical stage. The needle is then adjusted as follows: Place the arm of the needle in the groove of the carrier with the dissecting tip extending into the moist chamber. Push the plate of the carrier (*b*, in Fig. 2, page 126) over the arm of the needle and tighten the set screw *c* just enough to allow one to push the needle with a straight and even movement. The needle is now adjusted by hand so as to bring the tip within the field of a low power objective. The set screw is then tightened to clamp the needle in place. Further centering of the needle is made by the screws *e*, *f*, *g* and *h*, under the low and high powers of the microscope. Finding the tip of the needle under high powers is facilitated by using an ocular containing cross hairs.

The coverslip with its drop containing the tissue to be dissected is now inverted over the moist chamber. The cells to be dissected are brought into the field of the microscope. The tip of the needle is then raised by the screw *c* until brought into view first under low and then under high power. The apparatus is now ready for dissection.

In conclusion I wish to suggest to the prospective worker in this field that he use the utmost conservatism in his interpretations of observed effects produced in microdissection. One must constantly keep in mind the extraordinary instability ot living cell structures.

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BIOLOGICAL BULLETIN

THE LOCOMOTIONS OF SURFACE-FEEDING CATERPILLARS ARE NOT TROPISMS.

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Preliminary Observations.

While collecting cherries from a tree a large number of apple-tent caterpillars were thrown upon the ground. Later several of these were noticed climbing the trunk of the tree. They continued onward and upward; some to the top of the tree and others to certain branches. Near the tree was a rustic chair, the top of the curved back of which was separated from the trunk by about three inches. Several caterpillars climbed the back of this chair. When they reached the top, they would elevate the front portion of the body, wave it back and forth and around for a few minutes and then proceed down the opposite side of the curved arm. Sometimes the insect would turn about and retrace its steps. These observations suggested two questions. Are these caterpillars guided by a negative geotropism? If so, why did those that had reached the top of the chair descend?

Technique.

These experiments were performed upon a wooden vertical maze (Figs. 1, 2) and a copper horizontal one (Fig. 3); both of the open type. The former was used in the field and the latter in an out-door insectary.

The vertical maze (Figs. 1, 2) consists of a cylindrical post about four feet tall and an inch and a half in diameter, to which are attached five adjustable rectangular arms. Some of these arms are straight and unjointed; others are formed of three straight sections so articulated as to form an arm with three adjustable sections. The straight arms and the sections of the longer arms are all of the same length. The adjustments of the arms make it possible to set each and every one at any desired angle to the pull of gravitation. The lower pointed end of the post was erected in loose soil in such a manner that, by rotating the post on its axis, it was possible to vary the position of any arm with reference to either the direction of the rays of the light or the path of the wind.

The copper horizontal maze (Fig. 3), which was supported on slender glass pillars, had one of its central alleys connected with the supporting table by means of a cardboard incline two inches wide and thirty inches long. It was so located that the strongest light came from one side.

The point of view expressed in this communication was obtained by a critical study of 1,000 caterpillars of the apple-tent moth, *Malocosoma americana* Fab., 300 of the cabbage butterfly, *Pieris rapæ* Linn., fifty of the white tussock moth, *Memerocampa leucostigma* A. & S., and a few individuals of the young of each of a large number of species of butterflies and moths collected from the surfaces of a large variety of food plants. Throughout a summer practically every kind of surface-feeding caterpillar that a diligent search of the vicinity of St. Louis yielded was put to the test. Among those tested were the flat slug-like Lyncenidæ, the elongated Papilionidæ and Pyralidæ. and the fusiform Hesperiidæ; the naked Noctuidæ, the hairy Arctiidæ, and Liparidæ, and the bristly and spiny Ceratocampidæ and Nymphalidæ; the Notodontidæ with their erect anal somites and the Sphingidæ with their oblique stripes and caudal horns; the grotesque Saturniidæ and the looping Geometridæ.

Experiments with the Vertical Maze.

To facilitate description, the parts of the vertical maze were named as follows: The upright was called the post; each straight arm and each section of a jointed arm was given a distinctive letter. Thus (Fig. 1) the first arm above the ground was called *abc*, the second, *def*, the third *j*, the fourth *g* and the fifth *h*.

In the first few hundred experiments with the tent-caterpillars the arms of the maze were arranged in a variety of ways. In the latter experiments with them and in the experiments with all others the maze was arranged as in Fig. 2; but, the bands *G* and *H* were used only on special occasions. In some of the experiments the plain maze was used; in others one or two odorous bands of filter paper were attached to certain portions of the maze. In experimenting one or more caterpillars were placed on the ground at the foot of the post, or else upon the top of the post, or on some intermediate portion of the maze.

When placed on the ground at the foot of the plain maze, the tent-caterpillar almost invariably letisimulated. In the first few experiments with the tent-caterpillars, on recovering from the feint, the larva moved toward and up the post. If its initial movements were away from the post, after a few steps it turned about and moved towards the maze. This striking behavior recalled some experiments by which H. B. Weiss[1] discovered that certain water insects when turned loose several yards from their home, would immediately go directly to the pond. It looked as though these caterpillars were being mechanically led to the post. To test the matter, a series of experiments, extending over several days, were performed. In these experiments precautions were taken to see that the heads of the caterpillars pointed in a variety of directions. In some cases the majority of the larvæ moved towards the post, in others the majority moved away from the post, in yet others about as many went away from the post as toward it. Evidently the behavior noticed in the first experiments was merely a coincidence, the uniformity of which may have been due to some factor which escaped observation.

Once upon the maze, each caterpillar displayed marked individuality. It might continue all the way to the top of the post without mounting any of the arms; it might, on its way up, explore a few or all of the arms; or it might turn about before reaching the top and return to the ground. At intervals it was sure to pause and reach upward and outward with the front end of its body and move it, in a wabbly manner, from side to side. [For brevity throughout this discussion this form of behavior is called exploring movements.] Should it reach the top, it might encircle it, pausing now and then to make exploring movements; or it might rest there for a long stretch of time. However, it would be more apt to descend the post, pausing now and then to make exploring movements. It might continue along the post to the ground; but it would be more apt to make side trips to one or more of the arms. Often the same arm would be traversed several times. Finally it would rest for an indefinite length of time upon some part of the maze, or else reach the ground. Some caterpillars would leave the post permanently as soon as they reached the ground the first time. Others would remount the maze and explore it one or more times before leaving. The transcription of the field notes of two cases will illustrate the above description.

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[1] Notes on the Positive Hydrotropism of *Gerris marginatus* and *Dineutes assimilis*. Canad. Entom., Vol. XLVI., pp. 269–271.

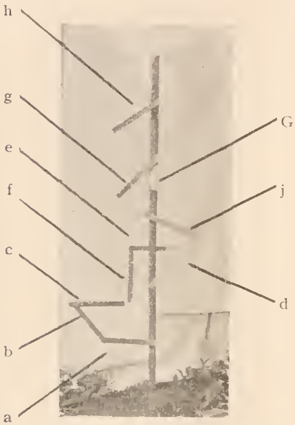

FIG. 1.

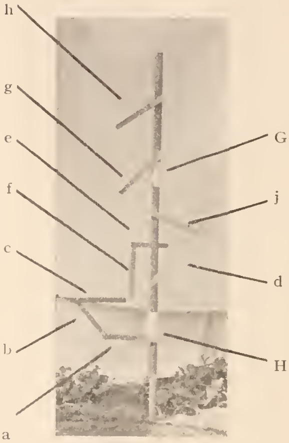

FIG. 2.

A tent-caterpillar about one inch long is placed on a maze arranged as in fig. 1; but without the band *G*. Ascending in a sinuous path, it passes around the bases of *a* and *d*, over the base of *j* and around the foot of *g* and *h* to the top of the post. The unhesitating manner in which it continues up and up is enough to rejoice any stanch believer in negative geotropism as the guiding power. Frequently pausing to make exploring movements,[1] it encircles the top a couple of times and then descends to *h*. It moves along *h* to its tip, makes exploring movements, returns to the post, descends to *e*, pausing several times to make exploring movements. It passes along *e* to *f*, makes exploring movements, descends *f* to its tip and makes exploring movements towards *c*. It reascends *f* to *e*, makes exploring movements, and returns to the tip of *f*. It makes exploring movements and then moves across the gap to *c*. It moves along *c* to *b*, along *b* to *a* and down *a* to the post. After making exploring movements, it encircles the post halfway, descends to the ground, moves off about three feet and rests indefinitely.

In this experiment, the subject of which is a tent-caterpillar, the arms *j*, *g*, *h* extend obliquely upward, on the left side of the post, at an angle of thirty degrees; *d* extends horizontally to the right; *e*, almost vertically upward, and *f*, almost horizontally back to the post; *a* extends obliquely upwards toward the left, at an angle of thirty degrees; *b*, horizontally towards the left; and *c*, obliquely upward, at an angle of about thirty degrees.

Following a path which is sometimes straight and sometimes spiral, the caterpillar continues upward until it reaches *g*, which it ascends to the tip. There it spends several minutes making exploring movements; then descends *g* to the post and crawls up it to the top. It goes about half way around the circumference, then crosses over to the opposite side and descends the post. Pausing occasionally to make exploring movements, it continues downward to *h*. It ascends *h* to its tip, crosses over to the opposite side and returns to the post. It continues downward an inch, rests a few minutes. Occasionally pausing to make exploring movements, it continues spirally downward to *g*, which it ascends for about four inches and upon which it rests quietly a few minutes. Returning to the post, it continues downward to *j*, makes exploring movements, moves up and down *j* several times and then returns to the post. In a sinuous line it continues down the post to *d*, which it mounts. Returning to the post, it continues downward to *a*, which it mounts, makes a few exploring movements and, returning to the post, continues downward to the ground. It then reascends the post a few inches and rests a few minutes. It continues upward to *d*, moves out *d* to *e* and rests thereon indefinitely.

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[1] When a caterpillar reaches the end of branch or twig, when it encounters an angle or an obstacle, and sometimes for no visible reason, it pauses, elevates the front portion of its body, stretches it forward and upward (sometimes slightly downward) and waves it with a wabbly movement. For the sake of terseness in descriptions, this form of behavior has been called "exploring movements."

If one may speak of types where there is so much individuality, the above descriptions may be considered typical of the many hundred of records. There were, to be sure, specific variations. Some species were sluggish and others rapid, some were vigorous, others fatigued quickly, some were persistently seeking a solution of the difficulty, others rested at frequent intervals and for long lengths of time, or else made practically no attempt to solve the problem; some letisimulated for a long period when placed at the foot of the maze, others began to climb at once. All these, however, are but minor variations, the essentials are as stated above.

When a caterpillar is placed on top of the post, either immediately or after a slight letisimulation, it moves down the post; making the same variety of random movements as are made by those caterpillars that are placed at the foot of the maze.

When a caterpillar is placed on one of the arms of the maze, either with or without a preliminary letisimulation, it soon reaches the post. There it may either descend or ascend and make the same type of random movements as is made by caterpillars placed at the foot of the maze.

As a variant, one or more bands of filter paper, saturated with oil of cloves or oil of cedar, were attached to the maze at *G* and *H*, as indicated in Figs. 1 and 2. When one scented band was used the caterpillar was placed either at the foot of the maze or on its top. Whenever the insect reached the scented band, it made exploring movements and then retreated. Otherwise its behavior was the same as that of a caterpillar on a plain vertical maze.

Where two scented bands were used (Fig. 2), the caterpillar was always placed on the maze between the two bands. The only way for the captive to escape to the ground, without crossing a scented band, was to cross the gap between *f* and *c* and proceed along *cba* to the lower portion of the post. Roaming at random to and fro over the maze, pausing from time to time to make exploring movements, retreating whenever it encountered a scented band, sooner or later the caterpillar would usually cross the gap between *f* and *c* and escape along *cba* to the lower post and thence to the ground. Occasionally a caterpillar would drop from the maze to the ground; in a few cases, after a few movements, it would rest indefinitely in one place; in less than one per cent. of the cases, the insect would escape across the scented band. In these cases the band had not lost its odor; for immediately thereafter another caterpillar, on reaching the same band, would retreat. Even the same caterpillar, on being replaced between the bands, would retreat from the band across which it had recently escaped.

A transcription of the record of one experiment will illustrate the behavior under these conditions. The specimen is a tent-caterpillar and the maze is arranged as in Fig. 2. The bands *G* and *H* are moistened with oil of cedar. It is between three and four in the afternoon and the maze is situated in the shade of trees; but, here and there, blotches of sunlight touch it. The caterpillar, which is placed near *j*, ascends the post until it reaches the band *G*. There it makes exploring movements and retreats down the post. On reaching *j*, it partly explores it, then returns to the post, and continues downward to *e*. It moves along *e* to *f*, makes a few exploring movements, descends *f* and crosses the gap to *c*. Returning to *f* it ascends *f* to *e* and then retreats to the lower end of *f*. After making exploring movements, it crosses the gap to *c*, moves along *cba* to the lower portion of the post and thence to the ground. *The caterpillar is returned to its former position between the two bands.* It descends to the band *H*, makes exploring movements and retreats up the post. It mounts *j*, and, after making exploring movements, returns to the post and descends to *H*. After making exploring movements, it encircles the post and ascends to *e*. It mounts *e*, then returns to the post and continues upward to the band *G*. After making exploring movements, it descends to *j*, makes exploring movements, returns to the post and continues downward to *e*. It moves across to *f* and back to the post. After ascending the post a short distance, it returns to *e*, passes along *e* to *f*, climbs down *f* to its end and makes exploring movements. It then ascends *f* to *e*, passes along *e* to the post and ascends it to the band *G*. After making exploring movements, it descends to *e*, passes along *e* to *f*, where it rests several minutes (in the bright sunlight). It then descends *f* to its end and reascends it to *e*. Then descending *f*, it passes across to *c*, passes along *c* to *b* and then retreats as far as *e*. It makes three trips back and forth between *e* and *f* and then passes from *e* to the post, and up it to band *G*. Avoiding the band, it encircles the post about three fourths its circumference and then falls to the ground.

With the tent-caterpillars these experiments were conducted either in the shade, or in shady places that were spotted with sunlight that had filtered through the trees. With many of the others the experiments were conducted in the bright sunlight. An accerelation of movements and an apparent tendency to climb or descend the shady side of the post were the only differences noted in the behavior of the caterpillars under these conditions.

EXPERIMENTS WITH A HORIZONTAL MAZE.

The maze used has (Fig. 3) been fully described in Vol. XXV, pp. 348–349, of the BIOLOGICAL BULLETIN. A cardboard incline thirty inches long and two wide connected one of the interior alleys of the maze with the supporting table. Eight inches of this incline extended above the maze (Fig. 3). This gave each caterpillar that arrived at the incline a choice of two pathways; one passing obliquely upwards, the other obliquely downwards. The maze was placed in an out-door insectary, all the light of which came through the netting covered north side. In the earliest experiments two caterpillars were used at a time, and they were so located that they would be forced to approach the incline from opposite directions. Later, when attention was focused upon the behavior of the caterpillar when coming in contact with the incline, twelve to fifteen insects were used at a time; and they were placed on various parts of the maze.

Although the insects displayed great individuality, the behavior in all cases was similar. They moved at random about the maze; roaming into blind alleys and out again, often retracing their steps when in the right pathway, and frequently pausing to make exploring movements. The rays of light were crossed at every possible angle. When a gentle breeze was blowing, there was not the slightest indication of an anemotropism. There was one striking uniformity. Whenever a caterpillar came in contact with incline for the first time, it practically invariably ascended it.[1] This happened when the contact was made where the edges of the maze and incline came in contact, and also when the exploring movements of the insect brought it in contact with some portion of the incline that was higher than the maze. After once mounting the incline the caterpillar

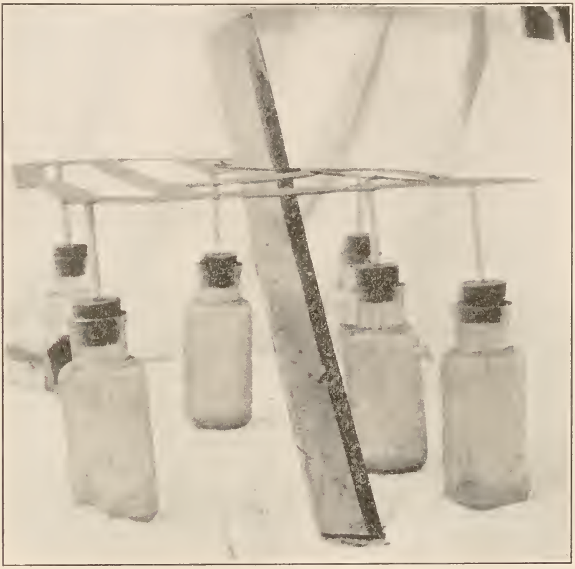

FIG. 3.

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[1] Only twice during the summer, did a caterpillar descend the incline upon its first contact with it.

would often return to the maze, roam around and then remount the incline. With a few exceptions, the caterpillar finally reached the table and departed. In a few cases it reascended the incline after reaching the table, and in yet fewer cases the caterpillar finally rested indefinitely upon some portion of the maze.

In spite of the individuality, there were four things common to the behavior of all of the caterpillars placed on the horizontal maze. (1) The caterpillars usually traveled along the edges of the maze, instead of along the middle of the roadway. (2) At frequent intervals the creature would stop, cling to the maze with its prolegs, stretch the front part of the body forwards and dorsalwards and wave it about in a wabbly manner. (3) On reaching the incline for the first time, it practically invariably moved upwards. (4) In all cases the locomotions were random movements. Any interpretation of the behavior on the maze must be in harmony with these four factors. The first and the third are the only ones suggesting that the reactions of these caterpillars are tropisms. The almost constant clinging to the edges of the maze suggests some form of a positive thigmotropism; the invariable ascension of the incline upon its first encounter seems to predicate a negative geotropism.

If the clinging to the edges of the maze is indicative of a positive thigmotropism, why does not the caterpillar show a pronounced tendency to linger in the many angles of the vertical maze? Why does it not rest indefinitely in the angle between the upper portion of the cardboard incline and that alley of the horizontal maze upon which it rests? Would it not be more consistent to believe that the caterpillars move along the edge because they secure a better foothold there than upon the smooth surface of the copper maze?

The fact that the incline was invariably ascended when first encountered requires critical analysis. An examination of the notes made at the time of the experiments shows that whenever the caterpillar upon the maze came in contact with the incline its head was directed towards its upper edge. A thoughtful consideration of the physical conditions of the horizontal maze used and of the normal movements of a caterpillar on the maze shows that such a condition is inevitable. Should a caterpillar be beneath the upper portion of the incline and elevate the anterior portion of its body to make the exploring movements which invariably accompany near contact with a change in its environment, it would be almost certain to touch the incline, and its head would inevitably be pointed towards the upper edge of the incline. Should the caterpillar approach the incline where one of its edges touches the maze and make the usual exploring movements what would happen? If it moved its uplifted body inwards it would come in contact with the incline and its head would be directed towards the upper edge of the incline. Should it move its body outwards, since the incline descends abruptly, the caterpillar would come in contact with nothing and would then wave its body inward. Should the sweep be sufficiently long, the caterpillar would surely come in contact with the incline and its head would be pointed towards its upper edge. This suggests that it is not necessary to predicate a negative geotropism to account for the upward movement of the caterpillar on its first contact with the maze. It simply moves off in the direction that its head happens to be pointing when it encounters the incline.

To test the soundness of this conclusion, the following simple experiment was devised. A caterpillar was placed in a long narrow test-tube; which was sufficiently wide to permit freedom of longitudinal locomotion, but which was so narrow as to make it inconvenienient for the insect to turn around or make exploring movements. This device made it possible to induce the caterpillar to come in contact with the incline with its head pointing in any desired direction. It was only necessary to wait until the caterpillar was moving freely along; and then, after first pointing the mouth of the test-tube in the desired direction, to bring it in contact with the upper surface of the incline. In more than 99 per cent. of the cases, the insect moved off in the direction its head was pointing.[1] By means of this device hundreds of caterpillars were induced to move downward, upon their first contact with the incline.

This also explains why a caterpillar approaching the vertical maze from the ground always moves upwards, while one placed on the tip of the maze always moves downward. It is not necessary to postulate a negative geotropism to explain one and a positive geotropism to explain the other, and puzzle our brains to account for the reversal of the tropic response when the insect is bodily transported from one place to the other. The behavior is not a tropism. When the caterpillar nears the foot of the maze, the exploring movements which it invariably makes when it nears a change in its environment would cause it to first encounter the post with its head pointed upwards. When placed on the top of the maze, if its movements bring it in contact with the side of the post, it will inevitably be with the head of the insect pointing downward. In one case the insect ascends, in the other it descends; in neither case is the predication of a tropism necessary to explain the behavior.

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[1] In the few exceptions, I had waited too long and the caterpillar had curved its head around the rim of the test-tube before I had brought it into contact with the incline.

Conclusion.

Evidently there is nothing about the behavior of surface-feeding caterpillars which warrants the assumption that their locomotions are tropisms. The movements made in locomotion are identical with those made by animals that learn by the trial and error method. Instinctively the caterpillars are physiologically attuned to a certain environment. Outside of that environment there is physiological unrest. To escape the unpleasantness of its environment, the creature makes random movements similar to those made by creatures that learn by the trial and error method. There is no automatic adjusting of the body so as to have it symmetrically stimulated by an external excitant. Some internal, instinctive stimulus causes it to move ahead until some sensation factor induces it to change its course. If physiological satisfaction is not obtained, it continues its random movements until fatigue causes it to rest. Evidently the locomotions of surface-feeding caterpillars need to be classified with trial and error movements rather than with tropisms.

It must be distinctly understood that nothing stated above refers to recently hatched caterpillars, nor to larvæ immediately before entering the pupa state, nor to caterpillars (like the coddling moth larva) which feed inside of plants. Their behavior is left for a future discusions.

FACTORS INVOLVED IN THE ATROPHY OF THE ORGANS OF THE LARVAL FROG.

WITHROW MORSE.[1]

From the Nelson Morris Memorial Institute for Medical Research of the Michael Reese Hospital, Chicago.

Preliminary.

Throughout the two great branches of the organic realm, instances are presented where persons, organs or tissues or cells themselves undergo retrogressive changes.[2] In the plant kingdom, we have the familiar elimination of stems, leaves and other parts of the individual, upon the approach of untoward conditions. In the animal series, we are enabled to identify an analogous process in the protozoa where, in *Amœba* the ephemeral pseudopodia are constantly being absorbed into the protoplasmic mass proper; in the species of *Actinophrys*, where this process of absorption is delayed permanently or at infrequent intervals; in the genus *Trypanosoma*, where the undulating membrane becomes absorbed under certain conditions. Similar phenomena are observed in the sponges where Maas's studies have shown that hitherto highly differentiated cells become reduced to a more typical and fundamental cell-form, the amœbocyte. In the Cœlenterata we find the hydranths of *Tubularia* (1) and of other species either absorbed or eliminated *in toto*, while in *Renilla* Wilson has described a degeneration of the polyp. The "brown bodies" of the Bryozoa represent degenerated individuals. In brachiopods, during the stages of fixation there is present an extensive degeneration of parts resembling in a superficial way the various changes undergone by the larvæ of the ascidians. In *Sacculina* we have, *facile princeps*, this property of involution. Even amongst the vertebrates we find instances of important degenerations of a natural sort, as in the fish *Fierasfer* where Bykowski and Nusbaum discovered extensive degeneration processes when the fish became parasitic.

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[1] In order to avoid confusion, the author's name will be used henceforth as in the title of this paper.

[2] Compare Child, C. M., "Senescence and Rejuvenescence." Chicago, 1915.

Interesting as these instances are, relatively meagre investigation has been made as to the causes and factors involved in the physiology of the process. On the other hand, such studies as have been made have borne good fruit. It was in the studies of ctenophore larvæ that Metchnikoff conceived the idea of what later became the theory of phagocytosis. Later this investigator studied the cytology of the larval frog's tail during the stages of metamorphosis. Extensive morphological studies have been made upon the stages of metamorphosis in insects. The changes which the salmon undergoes in breeding season have likewise received considerable attention.

Passing from the natural atrophy found in such instances as these, to what is termed pathological atrophy, we encounter the processes of progressive muscle atrophy, acute yellow atrophy of the liver and other states which bear many features in common with certain of the so-called natural atrophy processes, and this is true to such an extent in regard to the atrophy of the muscles of the larval frog at metamorphosis and the conditions observed in progressive muscle atrophy or Zenker's degeneration that it is difficult to believe that we are dealing with fundamentally different physiological processes.

The purpose of the following communication is to attempt to show that there are certain factors involved in the atrophy of the tadpole's tail which are to be considered as causal or of primary importance as conditions; and the similarity in cytological pictures and in certain known conditions in this case and in that of pathological atrophy makes it fairly probable that we are dealing with the same physiological factors, so that we encounter here an instance where the comparative physiology of a lower form of organism sheds light upon the economy of the higher organisms of especial interest to the pathologist.

HISTORICAL.

Although Metchnikoff had witnessed the activity of phagocytic cells earlier, in the larvæ of errantiæ, his first report involving the conception of cells wandering in the body fluids of one organism and serving as scavengers was made in 1883 (2). Early in the history of the problem, this investigator recognized the practical value of such a study when he says "L'atrophie des muscles des batraciens présente un grand intéret parce qu'elle peut servir de type de phénomenes pathologiques."

Following Metchnikoff, several investigators concerned themselves with the problem. Thus Barfurth (3), Looss (4), Noetzel (5), Anglas (6), Guieweisse (7), Mercier (8) are among the more important names in this connection. Of these, Looss and Mercier are perhaps of the greater importance. Grund (9) has more recently studied the problem of muscle degeneration from the chemical standpoint. In the field of fish physiology, Miescher's (10) classic researches and those of Noel Paton (11) and his co-workers demand especial mention. Even mere mention of the investigations which have been made in insect physiology and morphology during metamorphosing periods is impracticable in the present paper, a review of which being available in the paper of Mercier (8).

The Problem Stated.

Two quite diverse theories concerning the factors involved in muscle atrophy exist, namely, *phagocytosis* and *autolysis*. The former theory can best be stated in the words of Metchnikoff: "Von den ersten Stadien seiner Atrophie an, kann man in ihm eine gross Anzahl amöboider Zellen finden, in deren Innerin ganze Stücke von Nervenfasern und Muskelprimitivbündeln enthalten sind" (1883, p. 561). Looss adheres to the latter view in the following words: "Wir es hier nicht mit einer Degeneration, einer Entartung der Gewebe und ihrer histologischen Bestandtheil zu thun haben, sondern mit einer reiner Auflösung, mit einer Resorption im strengen Sinne des Wortes" (p. 91).

In the larval frog, no one has attempted to verify the belief of Looss save upon histological grounds. Anglas, Noetzel, Guieweisse and others subscribe freely to Metchnikoff's theory of phagocytosis. The burden of the present paper is to show that Looss is correct in believing that fundamentally and primarily, a change is initiated interpretable as autolysis and that phagocytosis, which unquestionably is present at a later stage, is of secondary importance.

HISTOLOGICAL EVIDENCE.

In the accompanying photomicrographs, evidence is presented that events have occurred in the tail of a larva before the advent of phagocytosis. Fig. 1 is that of a section through the larval frog's tail, lateral and ventral to the nerve and vertebral column. The frog was without any external indications of the approaching metamorphosis. However, the section shows that the process is under way, for there is a dissolution of the muscle bands, a loosening up of the fibers, and, in the larger spaces, phagocytes are to be seen in the act of engulfing portions of the dissociated musculature. The important point to be noted, however, is that there has been a change in the condition of the musculature in regions where no phagocytes occur. This change involves what has

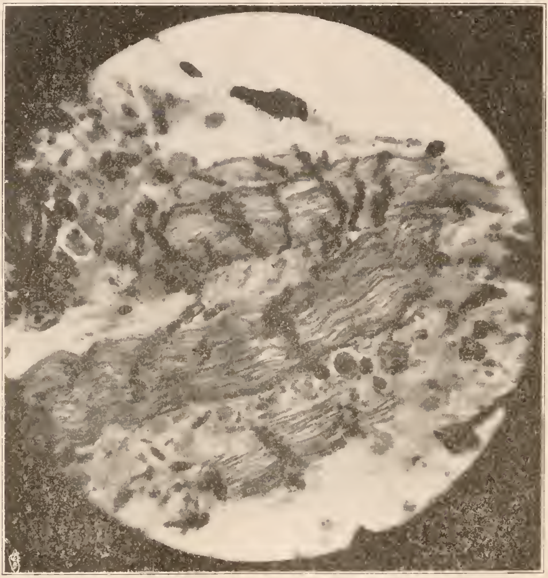

FIG. 1. Section through the tail of an involuting *Rana pipiens*. Zeiss 2 mm. obj. ocular 6. Osmium tetroxid fixation. Heidenhain's ferric alum-hematoxlyn.

been described as a "chromatolysis," but the dark masses running transversely over the muscle bands are depositions of fats bearing unsaturated fatty acids (oleic) which stain with the osmium tetroxid used in fixation. Willard and Guenther in unpublished investigations upon the micro-chemistry of degenerating muscle fibers find similar conditions in mammalian muscles permitted to degenerate after severing the innervation; a change in the distribution of neutral fats occurs involving the grouping of these compounds into masses, or droplets.[1] The significance in the present

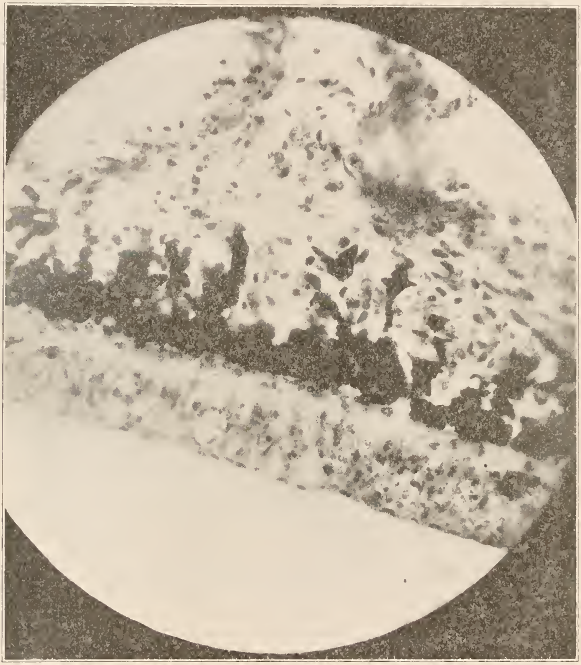

FIG. 2. Section of similar stage, same species, showing peripheral tissues. Not all of the intensely black areas are those of chromatolysis, for the osmium stains those neutral fats containing unsaturated fatty acids, such as oleic.

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[1] There is no evidence of an infiltration of fat, but rather of a simple process of accumulation of fats already present in diffused form.

instance is that whatever the process means, it is precursory to phagocytosis.

In the second figure, the same mobilization of the lipoids is to be seen in the layer immediately proximal to the epidermis. This does not appear in control organisms where the size of the individual together with the character of the cellular elements in the muscles and elsewhere negate the advent of metamorphosis. Accompanying the so-called but misinterpreted "chromatolysis" is a vacuolization of the tissues, an edema recognizable in the translucence of the tail when viewed in the individual intact and alive. This edema formation is to be seen in the second figure lying directly distally to the dark area. The vacuoles seen in the epidermis, however, are normal skin glands. Regarding, now, the question as to whether phagocytosis is a factor here, it must be admitted that there is nothing to indicate the presence of these agents. It is difficult to present this testimony in a black-and-white photomicrograph, but sufficient detail is given to insure that at least the process is not *pari passu* with the extent of edema formation and of lipoid mobilization. Perusal of the microscopic sections themselves makes it evident that no phagocytosis is operative in these tissues, although there have been some changes indicative of metamorphosis.

From histological evidence, then, we must accept the belief of Looss and others supporting him that phagocytosis as a primary event is not a fact, but that its participation in metamorphosis is a later and secondary event.

In passing, it is of interest to compare the histological pictures given by preparations of Zenker's or hyaline degeneration and those given by the normal atrophy of the larval frog's tail. The writer was first struck with this coincidence in examining the photomicrographs of Steiner's (12) article on dermatomyositis. From histological evidence and from physiological considerations it is probable that the natural atrophy of the metamorphosing tadpole is reflected in the pathological atrophy in cases of progressive muscle atrophy.

From the evidence thus far submitted it is probable:

1. That an event precursory to the appearance of phagocytes in the affected areas occurs.

2. That phagocytosis contrary to being a primary agent in effecting the dissolution of the tissues of the tail is of secondary importance.

Biochemical Evidence.

During the last few years, a more complete understanding of the softening of tissues in the living and in the dead organism without the participation of microörganisms has been obtained through the work of Bradley (13) and others. There is a more or less definite relation between reaction of medium and the process known variously as self-digestion, maceration, auto-digestion, autolysis, etc., wherein tissue enzymes resembling in a general way the enzymes of the alimentary tract operate in the tissues themselves, causing autolytic processes. This relation of reaction is such that when the average normal alkalinity of the tissues falls below a certain point, autolysis begins. In the terminology of physical chemistry, when the number of hydroxyl groups or ions (OH) approach (molecular weight in hydrogen equivalent)/(10,000,000) or $N/(10)^7$, which is neutrality for the ions involved, then either the proteolytic enzymes of autolysis are activated, or the reaction causes a change in the proteins rendering them digestible by the enzymes (14). A distinctly acid reaction, that is, where the concentration of OH ions is less than 1/10,000,000 normal, or, in the terminology of Sörensen which is being universally adopted, where $Ph < 7$[1], autolysis proceeds rapidly.

Up until the present time, the hydrogen ion concentration of the blood of the larval frog has not been accurately determined, a point to which the writer means to direct attention when material is available.[2] It is desirable to determine whether there is any change in alkalinity coördinate with atrophy. In the case of atrophy of the kidney tissue of the mammal where autolysis can be demonstrated, the writer and colaborator (15) have found that there is a rapid loss of alkalinity when autolysis sets in, while a true acidity develops later. If, in the larval frog, autolysis is *vera causa* for the process of atrophy, we should expect to find a low alkalinity or actual acidity of the body fluids of the tail during atrophy.

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[1] The expression *Ph* is borrowed from the principles of electromotive force, where the "potential" difference is measured on a potentiometer between a known concentration of hydrogen or hydroxyl ions and the unknown solution whose concentration in hydrogen or hydroxyl ions is required. The expression Ph signifies that the potential refers to hydrogen (h). Its numerical value is the same as the expression $N/(10)^a$, where a is a number greater than 7 if the solution in question is alkaline, or less than 7 if the solution is acid. Inasmuch as $N/(10)^a$ is the same as $N(10)^{-a}$, the expression *Ph* means "minus the logarithm of concentration."

[2] Only the larger species of frogs can be utilized for these determinations. The arva of *Rana catesbiana* is of sufficient size to give 2 c.c. of blood for experimental

If autolysis actually produces atrophy of the larval frog's organs, we should expect to find a difference in the quantities of end products of protein digestion between the normal, non-metamorphosing tail and that which is atrophying. However, the practical demonstration could not be considered especially easy since the work of Van Slyke and others upon mammals has shown that these end products, the lower polypeptids and amino-acids, are rapidly withdrawn from the blood by the various tissues. Even after a full meal, until the micro-methods of Folin, Van Slyke and others were brought into play, amino-acids in the blood could not be satisfactorily demonstrated. *A fortiori*, in the atrophying frog, large differences in amino-nitrogen in the two cases in question are not to be expected. Another point should be recalled in this connection: Muscle, of the various tissues, undergoes autolysis slowest, with the exception of nervous tissue.

The following experiment was designed to show whether, qualitatively, a difference in concentration of amino-acids could be observed between normal and atrophying tissues.[1]

Experiment.—The tail of a larva of the common frog, *Rana areolata*, was used. The larva selected had small posterior legs, anterior ones still beneath the integument and histological sections of similar stages of *Rana catesbiana* larvæ failed to show evidence of metamorphosis. The tissue was weighed to 0.5 gram wet weight, chipped fine and transferred to a Schleicher and Schüll No. 579

purposes, which is practically the lower limit for work such as the determination of acidity where at least a half cubic centimeter of serum is desirable.

[1] The writer reported, earlier (16) upon the concentration of amino-acids in the tissues of the larval frog, where direct determination by the method of Van Slyke was attempted; from further work it is evident that this evidence is of little value since the calculated amounts of amino-acid would be extremely small.

protein impermeable dialyzing thimble in which 1 c.c. of blood serum, free from hemoglobin, plus 2 c.c. of 0.7 per cent. NaCl solution had been placed. The thimble was suspended in 20 c.c. of distilled water, toluene added to both liquids within and without the thimble and placed in a thermostat at 30° C. over night. Similarly, tails from atrophying individuals were removed, and treated as the normal tail was prepared. At the end of 16 hours, the dialyzate in 2 c.c. amounts was boiled with three drops of 1 per cent. solution of "Ninhydrin" for one minute. The control (non-atrophying, normal) gave negative results while the atrophying tissue gave positive. Several duplicates gave similar results.

In interpreting this experiment, it may be urged that atrophying tissue digests more rapidly than normal tissue. To determine this point the following experiment was performed.

Experiment.—Sixty grams, wet weight, obtained by using the whole body of several non-metamorphosing individuals were ground in sand and made up to a 25 per cent. digest with toluene water. A similar digest was prepared from involuting larvæ. The preparations were incubated for five days at 37° C. Twenty-five cubic centimeter aliquots of the digests were pipetted into 50 c.c. tannic acid solution which was left to precipitate over night. The preparation was diluted to 100 c.c. filtered and 50 c.c. aliquots were examined by the Kjeldahl process for total non-protein nitrogen. Duplicates gave the following results:

Control	1.07 gm. protein per 60 gm. tissue.
Absorbing	1.68 " " " " " "

For the Van Slyke gasometric method, tannic acid could not be used to advantage as a protein precipitant; 50 c.c. of boiling saturated solution of potassium sulphate was made; otherwise the procedure was as before:

Control	110 mm. NH_2 nitrogen per 60 gm. tissue.
Absorbing	76 " " " " " " "

The formol-titration method gave:

Control	117 mm. NH_2 nitrogen per 60 gm. tissue.
Absorbing	95 " " " " " " "

Obviously, there is no acceleration, but rather an apparent inhibition, which is not a true inhibition of digestion rate, but is due to the fact that autolysis has proceeded in these tissues before the death of the larva, the products being withdrawn by the circulation, leaving only the slowly autolyzing tissues for this experiment. The apparent increase of 1.68 g. over 1.07 g. in the case of the non-protein nitrogen figure, is insignificant, while the difference in non-protein and amino nitrogen is due to peptones and other polypeptids.

The tissues, then, are not in more favorable condition for self-digestion in the case of atrophying than in normal instances.

The question arises as to whether the enzyme content varies in the two cases. This is improbable. Loevenhart (17) has shown that in liver atrophy in mammals, an ester splitting enzyme, esterase, is not increased over that of the normal liver. Bradley (18), again, has shown that in the mammary gland there is no increase in lipase during lactation over the resting condition of the gland. Finally, the writer (19) has interpreted his experiments upon atrophy similarly. However, the following experiment seems to demand that, if it is true that the atrophying tissues themselves do not digest faster *in vitro* than non-atrophying masses, there is something in the enzyme relations in the two cases which is different.

Experiment.—Four tenths of a gram of tissue, wet weight, from a non-involuting larva were placed in 3 c.c. of serum from an adult frog. Toluene was added. A control consisting of 0.4 c. of involuting material was made. Digestion was permitted at 30° C. for 24 hours. Each preparation was then diluted to 5 c.c. of fluid with distilled water. 2.5 c.c. aliquots were examined for amino-nitrogen by the gasometric method of Van Slyke:

Control	0.63	mm. NH_2 nitrogen per 0.4 g. tissue.
Absorbing	1.19	" " " " " " "

Here, the differences are approximately 1:2 and inasmuch as we have seen that there is probably more autolyzable material taken from the involuting tissue, which has undergone a certain degree of self-digestion or atrophy (compare the preceding experiment), leaving less to digest in artificial autolysis experiments, the difference is significant. There are two substrates present in this system, namely, blood-serum proteins and secondly the tissue from the tail. The conclusion is warranted that there is a difference in enzyme content between the atrophying and the normal tissue. Granting that the serum proteins are more readily digested by the enzymes of the tail issue, we are still driven to assume that the enzymes are different in the two cases.

We have seen, then, that correlative with histological differences, there are enzyme differences in the case of atrophying and non-atrophying material.

It is possible that the experiments described in the preceding paragraphs do not bear critically upon the problem as to what induces atrophy of the metamorphosing larva. It may be urged that phagocytes could be operative in each of these cases. To assume this, it would be necessary to demonstrate that within the relatively short period of 24 hours, phagocytes, working *in vitro*, could perform the task of digesting the tissue in the amounts given. We should at once seek to answer the question as to whether a marked increase of phagocytes is to be observed in atrophying material over the normal. The writer (20) has made differential counts of the blood cells of normal and involuting individuals with the result that no increase commensurate with the difference in digesting power in the two cases exists. That there is a concentration of phagocytes (polynuclear leucocytes) in the affected areas is evident from Fig. 1, given above, and also from the work of Mercier; the actual number, however, remains the same.

THE CAUSES INDUCING ATROPHY.

Mammalian experiments at the hands of Martin Jacoby and many other investigators have shown that atrophy involving autolysis is induced when the blood supply is interfered with. It is not necessary to occlude the supply directly; interruption of the blood supply will serve to cause atrophy. Bataillon (21) found that in the frog, the development of the pygostyle caused a change in the distribution of the blood supply throughout the tail. This does not involve complete occlusion, for Mercier found that, comparatively late in metamorphosis, phagocytes bearing carmin granules picked up from the dorsal lymph sac into which

Mercier had injected the grains, appeared in the muscle masses undergoing atrophy.

Granting that this explanation is adequate, we may seek the more immediate factor involved. The first suggestion may be that oxygen inhibits the activity of the autolyzing enzymes and that when the blood supply is interfered with, the inhibiting action of oxygen is thrown off, permitting autolysis. The experiments of the writer (22) speak decidedly against this theory of inhibition of autolysis by oxygen.[1]

The known relation between reaction of medium and autolysis, of which we have spoken earlier in the present paper is sufficient to afford an adequate explanation of what takes place at the inception of autolysis. Partial interruption of the blood supply everywhere results in an accumulation of carbon dioxid. The "buffer" value of the blood in alkalinity is soon neutralized and an actual acidity of the blood results ($Ph < 7$). Perhaps, likewise, as in starvation, acids other than carbonic acid enter the blood stream, such as those of incomplete oxidation—the so-called acids of acidosis, ketonic acids, beta-hydroxybutyric acid, aceto-acetic acid and the keton aceton. Even in the total exclusion of respiratory oxygen, intramolecular oxidation occurs, giving rise to carbon dioxid and to the acids of acidosis. This the writer takes to be the *modus operandi* of atrophy in the larval frog.

With regard to oxygen, the following experiment of the writer bears:

Experiment.—Several tall glass cylinders, 45 cm. high and 5 cm. in diameter were filled with water and into each was introduced a single larva which gave no evidence of metamorphosis. The larvæ were fed algæ,.but owing to the darkened space in which the experiment was conducted, photosynthesis, giving rise to oxygen, did not occur. The jars were left for several weeks, during which time the larvæ grew, but none of them exhibited any tendency towards metamorphosis. Controls in finger-bowls with oxygenated water metamorphosed.

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[1] The recent papers by Burge in the *American Journal of Physiology* contain the assumption of oxygen inhibition, but there is no experimental data to substantiate the theory.

Experiment.—Tap water was boiled and cooled in hermetically sealed jars. Finger-bowls were filled with the water and larvæ introduced. There was a high mortality amongst the larvæ, but a few were kept for two weeks, during which time no metamorphosis occurred.

Experiment.—The dorsal nerve cord together with the aorta was ligated with silk thread, at the base of the tail. Again, the mortality was high, the open cut being attacked by *Saprolegnia* and water moulds, wild yeasts, etc. No acceleration in metamorphosis was observed.

Lack of oxygen, or a marked reduction in its amount does not induce autolysis in the larvæ. It must be admitted, however, that the negative results of the last experiment do not bear out the theory that interference in the blood supply induces atrophy. Lateral circulation, however, may have been a factor.

Wintrebert (23), seeking the cause of atrophy in the larval frog, suggested that an internal secretion induces metamorphosis. The following experiment was directed towards the obtaining of evidence upon this point:

Experiment.—Five normal larvæ were isolated in large petri dishes. Serum was injected into the dorsal lymph sacs of these larvæ, the serum having been obtained either from the blood of involuting larvæ or from the expressed juices of the tail. No attempt to keep the preparation sterile was made owing to the obviously impossible nature of the task. The results were negative as far as inducing metamorphosis.

Attention has been directed to the glands of internal secretion. Thus Babák (24) has studied the effects of injections of hypophysis and of other portions of the brain. Positive results were obtained which this writer interpreted as due to direct effects upon the nervous system rather than upon the tissues. Babák considers that there is a hormone-action involved in the sense of Bayliss and Starling. The thyroid has received a relative large amount of attention. Gudernatsch originally observed a precocious metamorphosis in the case of larvæ fed upon thyroid preparations and since his work, others have studied the problem from the biochemical aspect. David Marine (25), Lenhart, the writer (26) and recently, from a more general physiological aspect, Allen (27) have examined the relations of the thyroid and thyroid components upon metamorphosis. The writer found that an iodized amino-acid (3–5-di-iodo-tyrosin, $C_6H_5OHI_2\,CH_2.CH.NH_2.COOH$), induces metamorphosis in the frog's larvæ, this amino-acid being derived from the thyroid tissue by means of acid hydrolysis and hence being a normal component of the thyroid. These observations taken in connection with the interesting morphological studies of Allen give us a *rationale* of the rôle of the thyroid in inducing metamorphosis. When thyroid tissue or its components is fed to an organism, the general metabolism is increased as evidenced by the increased output of the end products of protein, carbohydrate and fat metabolism. When thyroid is removed from the economy of the organism, as in Allen's thyroidectomy experiments, metabolism is decreased; this is indicated by his further observations that reduced nourishment obtains the same results, that is, in both cases metamorphosis is suppressed or delayed. We may conclude, then, that metamorphosis involves, as one of its principal factors, heightened metabolism.

It is necessary to distinguish between heightened metabolism, which involves only *catabolism*, in Gudernatsch's experiments and the accelerated anabolism present in Allen's cases. In both instances, metabolism is heightened, but the current is down stream in the one case, leading to loss of storage materials and a piling up of materials in the form of fat, etc., in the second case. Now it is of interest to recall that according to Voegtlin and Strouse, the iodized amino-acid which was found to operate as whole thyroid tissue in inducing precocious metamorphosis, fails to replace the function of thyroid in those pathological cases where there is a deficiency in thyroid function. It would be of considerable interest to determine whether 3–5-di-iodo-tyrosin, thyreoglobulin or any of the chemical components of the intact thyroid tissue replaces the absent thyroid in cases of thyroidectomy in inducing metamorphosis, for wrapped up with the question is the important and fundamental one as to what relation the iodine has in the thryoid. If the iodized amino-acid should in itself, in the absence of the thyroid, induce metamorphosis, it would show that the theory that iodine stimulates an internal secretion in the thyroid gland, rather than functioning as hormone itself, is untenable. In either case it would clear up the apparent discrepancy in the findings of Voegtlin and Strouse and in those of the students of amphibian metamorphosis.

THE ECONOMY OF THE PROCESS.

In the investigations upon the salmon conducted by Miescher and later by Noel Paton and others, it is evident that there is a high degree of economy involved in the process of metamorphosis of the muscles into gonad material. The question arises, is this true for the metamorphosis of the larval frog?

In the foregoing paragraphs, we have spoken of this process of metamorphosis as if it were confined to the regions of the tail, but the difficulty of studying the participation of the alimentary tract, gills, etc., in the process is not a small one and these investigations have been confined to the changes in the tail. One primary difficulty regarding the study of the alimentary tract is that at metamorphosis, preceding the shortening of that system, a large accumulation of fecal matter is expelled, containing the same chemical compounds which are utilized in studying the transformations of tissues. It is for this reason that the simple expedient of weighing the organism before and after metamorphosis to determine the loss, if any, of material, is not feasible, the fecal matter far overbalancing any amount of product of tissue change thrown to the outside.

A study was made of the excretions of the larvæ immediately after the expulsion of the fecal matter. Larvæ were isolated in distilled water in large petri dishes. After twelve hours, the water was filtered and estimations were made of the total nitrogen, amino-nitrogen, ammonia and urea. While the method was crude, owing to the impossibility of inhibiting the transformation of urea into ammonia and of the probable deaminization of the amino-acids by bacteria, there was no constant difference in the figures for non-metamorphosing and metamorphosing lavræ. Unless, then, there were extensive excretion of the products of the decomposition of the larval tissues, it is safe to conclude that the process is an economic one, the tissues of the larval organs contributing to the formation of those of the adult.

Pointing to the correctness of this interpretation is the fact that during the development of the adult organs (legs, etc.) no food is taken by the larva. Storage of fat, etc., has taken place during the earlier period of larval existence, but the important thing is to remember that *protein is never stored as such in the animal organism.* The nitrogenous material for the legs, etc., developing during metamorphosis must be supplied from some source other than storage. The simplest explanation is to assume that it is derived from the larval organs.

VON BAER'S LAW AND LARVAL METAMORPHOSIS.

The frog has been taken as a classic example of the operations of the so-called Law of Von Baer, termed sometimes the Law of Recapitulation and at others that of Repetition. The question is, can the metamorphosis be explained wholly upon the assumption that physico-chemical events other than those concerned with heredity initiate the process?

Those who have experimented with the agents accelerating metamorphosis have found that these compounds are impotent except in the case of larvæ which have reached a certain period of their existence. In other words, we know of no agent which is operative regardless of the stage of development of the larvæ. We may suppress metamorphosis; we can not, or have not induced it in stages far removed from those in which it would normally occur. This indicates that a certain cycle of events, probably determined by heredity, are necessary before any stimulating agent is effective. The growth of the pygostyle according to Barfurth, initiates the process of atrophy in the tail, but what events stimulate the growth of the pygostyle are unknown. In some species, which do not metamorphose during the first summer, the larvæ are subjected to relatively the same stimuli from the environment during the second summer as during the first. It is improbable that external conditions determine the time of metamorphosis. Again, to offer the explanation that hormones or enzymes initiate metamorphosis leaves open the more potent question as to what determines the development of these compounds.

CONCLUSIONS.

1. Autolysis is the primary factor in the physiology of atrophy in metamorphosis.

2. This process is probably induced by an acidosis in the tissues.

3. Adopting Barfurth's suggestion of the occlusion of the blood vessels at the base of the tail by the growth of the pygostyle, it is probable that carbon dioxid and acids of incomplete combustion accumulate and render the reaction of the blood less alkaline or more acid.

4. Phagocytosis is not a primary factor, as Metchnikoff believed, but a secondary one, the phagocytes being attracted by chemotaxis to the atrophying organs.

5. The process of metamorphosis is economic, the substances derived from the atrophying organs being utilized by the growing adult organs.

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STUDIES IN THE PHYSIOLOGY OF SPERMATOZOA.[1]

EDWIN J. COHN.

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[1] From the Marine Biological Laboratory, Woods Hole, Massachusetts, and the Hull Zoölogical Laboratory, The University of Chicago.

I. Introduction.

The changes in the physiological condition of the spermatozoon, from the time it is extruded from the genitalia of the male until it "undergoes the transformation into a nucleus" (Loeb, J., 1913, p. 306) in the protoplasm of the egg are dependent in rate upon environmental conditions. The germ cells of most marine invertebrates are extruded into sea water, and fertilization of the egg by the sperm there follows. The environment, sea water—or sea water modified by the excretions of the egg or of the sperm—must therefore be studied in order to understand the variations in the physiological condition of spermatozoa that have often been observed.

This investigation had its beginning in an attempt to understand seemingly contradictory effects of sea water that had contained the eggs of the sea urchin,[1] *Arbacia punctulata*, upon the activity, the length of life and the "fertilizing power" of the spermatozoa of the same species. For interesting me in these phenomena, and for invaluable aid in this attempt at their solution, I am indebted to Dr. F. R. Lillie.

The experimental work was carried on during the summers of 1915 and 1916 at the Marine Biological Laboratory at Woods Hole, Massachusetts. During that time the behavior of the germ cells of other marine invertebrates were sufficiently observed to suggest that the relations that are hereafter reported for *Arbacia* are not highly specific.

Material and Methods.

The sperm of the sea urchin, *Arbacia punctulata*, are shed from the genital pores, if the peristome is cut, and the urchin placed aboral side down. The sperm may then be collected in a clean dry watch glass, and diluted to any concentration by the admixture of sea water. The concentration of the sperm suspension records the extent of dilution of the sperm. A one per cent. suspension is one in which one drop of sperm is added to 99 drops of sea water or of sea water that has suffered a definite modification.

The relative "fertilizing power" of sperm that had been subjected to different environments was determined by adding such sperm in identical concentration to the ripe eggs of the same species in sea water at different times. The average number of eggs that subsequently developed was estimated by counting at least a hundred eggs at about the four cell stage.[1]

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[1] Such sea water is for convenience called egg water.

In tabulating the experiments that are reported (in Tables I., II. and III. the concentration of spermatozoa was the variable environmental condition) the concentration of spermatozoa in the suspension is recorded in a column at the extreme left. The variable environmental conditions that obtained in the different suspensions are recorded in a legend over the columns representing the percentage of eggs that were fertilized when a definite number of drops of the sperm suspension (the number of drops of sperm is reported either in the legend or in a column at the extreme right) were added to a constant quantity of eggs in a given amount of sea water at the intervals noted.

The effects of change in environmental condition upon sperm were under investigation. Since the influence of change in environmental condition upon eggs and upon sperm are not dissimilar (Loeb, J., 1913, Robertson, T. B., 1912) the variation in the physiological condition of eggs (Gemmill, 1900; Vernon, 1899; Loeb, J., 1913; Goldfarb, A. J., 1917) was eliminated by always inseminating in sea water.

For the same reason, the eggs of but one female were used in each series of inseminations. The fertilizing power of different sperm suspensions were in this way tested. The eggs were obtained by straining the cut up ovaries through cheese cloth into sea water, and subsequently washing the eggs by decanting the supernatant fluid. Eggs were never used after they had been in sea water for more than six hours. The forceps that were employed in removing the ovaries were never used for any other purpose. If a male had previously been opened, the hands and the scissors with which the peristome had been cut were rinsed in fresh water. A control of unfertilized eggs was always kept, but no contamination was ever observed.

The failure of sperm to fertilize ripe eggs may be employed as an indication of death only if accompanied by some other observation such as the dissolution of the protoplasm that follows the death of spermatozoa. This is necessary, since a decrease in the activity of spermatozoa may also decrease their fertilizing power. But sperm that are non-motile can be reactivated, and until reactivation is no longer possible, sperm can not be considered dead.

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[1] See Lillie, F. R., 1915; Fuchs, H. M., 1915, for a detailed description of this procedure

The rate of movement of spermatozoa cannot, however, be observed with any degree of accuracy. While it is not difficult to distinguish between a very motile, a fairly motile and a non-motile sperm suspension, more delicate fluctuations in spermatozoön activity cannot easily be observed. As in other cells a delicate indicator of the degree of activity is afforded by the measurement of the oxygen consumption (Loeb and Wasteneys, 1912; Warburg, 1910) the heat production (Meyerhoff, 1911) or the carbon dioxide production of a sperm suspension. The carbon dioxide production of sperm suspensions of different concentration has been measured.

The activity of spermatozoa, as will presently appear, is effected by changes in either the temperature, the osmotic pressure or the hydrogen ion concentration of sea water. Since even the carbon dioxide produced by spermatozoa is sufficient appreciably to change the hydrogen ion concentration of a 0 005 per cent. suspension, since the activity of spermatozoa is a function of the hydrogen ion concentration, and since the fertilizing power of a sperm suspension is related to the activity of the spermatozoa, the careful control of the environment becomes a necessity.

Sea Water as Environment.

The environment of the spermatozoa of the sea urchin is sea water. A variable in sea water that is known to effect the activity of spermatozoa is the hydrogen ion concentration. The concentration by weight of hydrogen ions in sea water is approximately 0.00000001 N or 1×10^{-8} N (Palitzsch, Sven, 1912). In a neutral solution there are, by definition, as many hydrogen as hydroxyl ions. The concentration of hydrogen ions in a neutral solution is 1×10^{-7} N or 10×10^{-8} N. There are therefore in the neighborhood of ten times as many hydrogen ions in a neutral solution as there are in sea water and ten times as many hydroxyl ions in sea water as there are in a neutral solution. Since the acidity or the alkalinity of a solution is measured in terms of its concentration in hydrogen ions, sea water is appreciably alkaline. The hydrogen ion concentration is often expressed as the negative logarithm to the base 10. This is called the hydrogen potential (Ph) (Sörensen, S. P. L., 1909). The hydrogen potential of sea water is therefore Ph 8. During the months of July and August, 1916, the sea water at the Marine Biological Laboratory at Woods Hole only varied between Ph 7.95 and Ph 8.15.

The hydrogen ion concentration of sea water was measured by colorimetric comparison with solutions of borates and phosphates. These were always standardized with a concentration cell. The indicators phenolphthalein, naphtolphthalein and neutral red satisfactorily covered the range investigated. Corrections for the effect of salts upon these indicators have been determined (Sörenson & Palitszch, 1910, 1913; Palitszch, 1911) and were employed.

The hydrogen ion concentration of "sea water is fully determined by (1) the tension of carbonic acid, (2) the concentration of water, or salinity, and (3) the temperature. This relationship suggests a method of determining the carbon dioxide tension of sea water" (Henderson, L. J., and Cohn, E. J., 1916, p. 620), for the effect of salinity upon the hydrogen ion concentration has been found to be very small (Henderson, L. J., and Cohn, E. J., 1916; McClendon, Gault and Mulholland, 1917). The effect of the temperature has been determined (Henderson, L. J., and Cohn, E. J., 1916; McClendon, Gault and Mulholland, 1917), and the relation between the hydrogen ion concentration and the carbon dioxide tension of sea water at a temperature of 20° C. has been reported by Henderson & Cohn (1916); at a temperature of 30° C. by McClendon (1916 and 1917). The measurement of the carbon dioxide tension of sea water (that is the partial pressure of the gas that is in equilibrium with sea water containing a definite concentration of carbon dioxide) can therefore be made with great accuracy and great rapidity. The carbon dioxide tension is recorded in terms of the number of millimeters of mercury that represents the partial pressure of the gas in the atmosphere at a total pressure of 760 millimeters, and a temperature of 0° C.

The change in the carbon dioxide concentration of sea water is not proportional to the change in the carbon dioxide tension, for increase in the carbon dioxide tension is correlated with a change in the equilibrium between the normal carbonates and bicarbonates in sea water. It has been calculated (Henderson, L. J., and Cohn, E. J.) that the former are converted into the latter at exactly the tensions of carbon dioxide that obtain in the ocean. At tensions of carbon dioxide greater than these an increase in tension may, as a first approximation in determining the carbon dioxide concentration, be considered as an increase in free carbonic acid. A "conversion table" for determining the carbon dioxide concentration (or content) of sea water has been published by McClendon (1917). The measurements of the total carbon dioxide concentration upon which this "conversion table" is based are not reported. Exact data defining the relation between the hydrogen ion concentration and the carbon dioxide concentration of sea water are therefore still unknown.

II. Environmental Conditions that Affect the Activity of Spermatozoa.

That "all the phenomena connected with the origin and death of the spermatozoön seem to be in accordance with the view, that its motion is essential to its function" (Newport, G., 1853, p. 261) was the opinion of the early investigators of the rôle of the sperm in fertilization. Indeed so completely was the movement of the "spermatic animalcules" found to depend upon temperature (Spallanzani, quoted from Newport, 1851, p. 235) (Prevost and Dumas, 1824) (Newport, G., 1851); osmotic pressure (Koelliker, A., 1856) and hydrogen ion concentration (Koelliker, A., 1856) that the observed activity of these cells was for a time supposed to be due to Brownian movements.

"Es fällt somit die Theorie, die Bewegung der Samenfäden sei willkürliche thierische Bewegung, haltlos zusammen. Welche physikalischen Kräfte aber dieses Phänomen erzeugen mögen, ist noch völlig dunkel. Ja wir können noch nicht einmal mit Bestimmtheit behaupten, obwohl diess wahrscheinlich ist, dass die Samenfäden auch im Organismus, im Hoden oder in den weiblichen Genitalien sich bewegen, es kann Niemand mit Bestimmtheit widerlegen, dass nicht etwa diese Bewegungen erst in den aus dem Organismus entfernten Objecten unter dem Mikroskop, als ein Analogon der Brown'schen Molecularbewegung entstehen, sei es durch Verdunstung oder irgend eine andere physikalische Ursache. Es ist mehr als wahrscheinlich, dass die Bewegungen wenigstens in einer physikalischen Wechselwirkung zwischen Flüssigkeit und Samenfäden begründet sind, wofür schon die ausserordentliche Abhängigkeit der Bewegungen von der Concentration und Beschaffenheit der Flüssigkeit, ferner vor Allem die Abänderung der Bewegungsacte durch Zusatz von Wasser, die Abhängigkeit der Art der Bewegung von der Form der Samenfäden der verschiedenen Thiere spricht, Umstände, welche auch auf andere Weise als durch einfache Adhäsionsverhältnisse, Vermehrung und Verminderung des Widerstandes zu wirken scheinen" (Koelliker, A., 1856, p. 202. Quotation from Funke im Lehrbuch der Physiol. von Günther, Bd. II., Abth. IV., 1853, p. 1027).

Dissenting from this position Newport "regarded this motion as being only the visible indication of a peculiar force, or form of vitality, in the impregnating agent, the spermatozoön, by which it is destined to arrive at, and is to expend on the object to be fecundated, and the effect of which is to strengthen, to augment, and possibly also to modify the nature of the formative changes, which are going on in the yet unimpregnated egg, per se; but which will subside, and soon entirely cease, if not reinforced through the agency of the spermatozoön" (Newport, G., 1853, p. 260). And again: "Whatever be the relation of this motion to its peculiar faculty, it is evident that motion is intimately associated with, and dependent on, its material composition and structural development" (Newport, G., 1853, p. 261).

Temperature.

The environmental conditions that effect the behavior of the spermatozoön were therefore abundantly and carefully observed by early investigators. "Spallanzani found that the fluid of the fœtid terrestrial toad (*Bufo calamita?*) at a high temperature of the season, 70° F. to 73° F., at which this species spawns in Italy, had lost its fecundatory influence at the end of six hours; but that in the temperature of an ice house, 40° F., it retained its efficacy for 25 hours" (Newport, G., 1853, p. 235). Observations of this kind were repeated by Prevost and Dumas (1824) and Newport (1851 and 1853) who wrote: "The general conclusion which seems to be deducible from a comparison of the observations of Spallanzani and of Prevost and Dumas, with those by myself, in regard to the tail-less Amphibia, is, that . . . the vitality of the spermatozoön, and the duration of its fecundatory power, are in a ratio inverse to that of an increase of temperature in the surrounding medium" (Newport, G., 1853, p. 237).

Hydrogen Ion Concentration.

Nor was temperature the only environmental factor that was known to effect the physiological condition of the spermatozoön. In a classic paper, "Physiologische Studien über die Samenflüssigkeit" (Koelliker, A., 1856), Koelliker demonstrated that the effect of substances supposedly "harmful" to the activity of the sperm, disappeared if the solutions were first made isotonic and isohydric with the suspension. He understood that a slightly acid solution might inhibit the activity of the sperm without killing them, and that reactivation followed upon neutralization of the acidity. He observed that if KOH, Na_2HPO_4 or blood were added to suspensions of paralyzed spermatozoa motility was recovered.

That the degree of activity is a function of the hydrogen ion concentration of the sperm suspension has been confirmed by subsequent investigations. In 1907 Günther (Günther, G., 1907) showed that not only could sperm be reactivated by a decrease in the hydrogen ion concentration, but also that they could be inactivated by an increase in the hydrogen ion concentration. He noticed that if a weak electric current is passed through a mammalian sperm suspension the sperm congregate at the positive pole and are there inactive. The hydrogen ion concentration is greatest at the positive pole. If the current is reversed sperm that have been inactive at the positive pole recover their activity and accumulate at the other end of the suspension; now become the positive pole. There they are again inactive. In the more alkaline part of the suspension the sperm are extremely active. Frog sperm (Lillie, R. S., 1903) and Echinid sperm (Gray, J., 1915) behave in a similar manner. This phenomenon does not occur if sperm are first inactivated by an increase in the hydrogen ion concentration of the suspension (Gray, J., 1915).

Oxygen and Carbon Dioxide Concentration.

Similar in kind is the observation of Buller (Buller, A. H., 1902) that when a bubble of oxygen is incorporated in a suspension of *Echinus* sperm those in the immediate vicinity of the oxygen remain active after the sperm at a greater distance "have all come to rest from want of oxygen." Between the active sperm and the inactive sperm there is a zone "in which there are comparatively very few spermatozoa." The active sperm gradually traverse the clear zone "and collect on the inner edge of the zone" (of inactive sperm) "upon reaching which they cease to move. A ring of thickly placed, dead spermatozoa thus arises" (Buller, A. H., 1902, p. 158). The spermatozoa were not "dead" but merely inactive in a region of low oxygen and of high carbon dioxide concentration.

F. R. Lillie has observed the converse phenomenon. He injected a drop of sea water with a carbon dioxide concentration of approximately 1 per cent. into a suspension of *Nereis* sperm (the sperm of *Arbacia* are not so sensitive to carbon dioxide but "the reactions of *Arbacia* spermatozoa are essentially the same in principle as those of *Nereis*") (Lillie, F. R., 1913, p. 546) and noticed that a ring of sperm is formed at a definite concentration of carbonic acid. This ring is separated by a clear zone from the active sperm in the rest of the suspension. "If the external edge of the clear zone be carefully observed, the spermatozoa can be seen to detach themselves one by one from the general suspension and pass straight over to the ring" (Lillie, F. R., 1913, p. 535).

It is apparent that increase in the hydrogen ion or in the carbon dioxide concentration or decrease in the oxygen concentration[1] decreases the activity of spermatozoa, while decrease in the hydrogen ion or carbon dioxide concentration or increase in the oxygen concentration increases the activity of spermatozoa. Three explanations of the configuration that appears when spermatozoa are subjected to such a gradient in carbon dioxide as has been described have been suggested; (*a*) that spermatozoa are activated in certain concentrations of carbon dioxide; (*b*) that spermatozoa are positively chemotactic or chemotropic to carbon dioxide; (*c*) that the accumulation of spermatozoa at a certain concentration of carbon dioxide is brought about by their inactivity in that concentration of carbon dioxide.

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[1] The observations of Drzewina, A., and Bohn, G. (Drzewina, A., and Bohn, G., 1912) upon the effects of lack of oxygen upon the length of life of spermatozoa will be considered in another place.

While it is not inconceivable that spermatozoa are activated in certain concentrations of carbon dioxide, no evidence of such a primary stimulation of spermatozoa has ever been observed or reported.

The circumstances which led to the formulation of the second hypothesis are quite intricate and will require an historical introduction if the problem is to be understood. Ever since Pfeffer "demonstrated the importance of the part played by chemotactic stimuli in causing the spermatozoa of liverworts, mosses, ferns, etc., to approach the oöspheres" (quoted from Buller, A. H., 1902, p. 145) biologists have tacitly assumed or attempted to demonstrate that this chemotactic phenomenon is general in fertilization not only in plants[1] but in animals. In 1895 Bergh suggested that "the spermatozoa collect around the ripe eggs, probably attracted by a special substance "(quoted from Buller, A. H., 1902, p. 146). Three years later Massart (Massart, J., 1888) demonstrated that the spermatozoa of the frog were positively thigmotactic to glass. He was, however, unable to demonstrate chemotaxis. This observation had previously been made by Dewitz (Dewitz, J., 1886). Massart also maintained that spermatozoa were positively thigmotactic to agar and gell (Massart, J., 1888) especially that of the egg (Massart, J., 1889).

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[1] There is some doubt that chemotaxis is a general phenomenon in the fertilization of plants. To a recent study of the "Physiology of Fucus Spermatozoids" the following summary is appended. "Using the Pfeffer capillary tube method of determining chemotaxy, it was found that certain acids cause collection of *Fucus* spermatozoids. It is suggested that this may be explained as due to toxicity and not chemotaxy" (Robbins, W. J., 1916, p. 130).

In 1900 Buller observed the "agglutination" or "cluster formation" of *Arbacia* sperm in water that had contained eggs of the same species and suggested that "a tactile stimulus appears to play a part in the phenomenon" (Buller, A. H., 1900, p. 387). As the "aggregation" of sperm by carbon dioxide has been supposed to be due to chemotaxis, so also has the "agglutination" of sperm by "egg water" (de Meyer, J., 1911, Lillie, F. R., 1913; Glaser, O., 1914). The phenomenon of "agglutination" will be discussed in another place. The configuration of *Arbacia* sperm in "agglutination" and of *Nereis* sperm in "aggregation" is not dissimilar (Lillie, F. R., 1913).

In 1902 Buller described the accumulation of inactive sperm in an oxygen gradient. This has been characterized as the converse of Lillie's "ring formation" in a gradient of carbon dioxide (p. 12). Buller explained the phenomenon as the result of the differential activity of spermatozoa in the gradient. He was, however, unable to account for the "clear zone" that occurs in the configuration. In consequence subsequent workers have had recourse to the accessory hypotheses that have been discussed. While there is no a priori reason for believing either that spermatozoa are not activated in certain concentrations of carbon dioxide or that they are not chemotactic to carbon dioxide, it should be pointed out that no positive evidence for either assumption has ever been demonstrated.[1]

III. The Relation between the Activity and the Longevity of Spermatozoa.

Increase in the activity of spermatozoa leads to a decrease in the length of time during which spermatozoa exhibit activity. This was observed by Koelliker, who remarked, regarding the effect of the alkali salts of carbonic acid, that they behaved much as the caustic alkalies in that: "Sie erregen die Samenfäden lebhaft, doch dauert deren Bewegung nicht lange" (Koelliker, A., 1856, p. 239). Koelliker emphasized that this activation occurred only in weakly alkaline solution, since sperm were "injured" in more concentrated alkaline solution.

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[1] "Dewitz, Buller, and the writer have vainly tried to prove the existence of a positive chemotropism of spermatozoa to eggs of the same species" (Loeb, J., 1916, p. 93).

From these and other observations Koelliker came to believe that the nourishment and therefore the length of life of the spermatozoön after it is liberated from the testes is dependent only upon the material of which it is constituted. "Eher wäre daran zu denken, ob nicht vielleicht die Körper der Samenfäden sich zu den Fäden selbst verhalten, wie eine Zelle zu ihren Wimperhaaren, und dieselben aus dem in ihnen enthaltenen reichlichern Material ernähren, eine Vermuthung, die jetzt, wo ich zeigen kann, dass die Samenfäden aus den Kernen der Samenzellen sich bilden, wohl ausgesprochen werden darf. Zu erforschen ist auch noch, ob die Samenfäden bei ihren Bewegungen elektrische Ströme entwickeln, und ob sie nicht, so lange sie sich bewegen, CO_2 abgeben, während sie O aufnehmen, Verhältnisse, über die ich vielleicht später berichten kann" (Koelliker, A., 1856, p. 245).

On the basis of observations of a quite similar kind Gemmill (1900) came to exactly the opposite conclusion. He observed that "the term of vitality of spermatozoa varies according to the degree of their admixture with sea water" (Gemmill, J F., 1900, p. 170) and correctly concluded that "mixing with sea-water stimulates the activity of movement of the spermatozoa, which become the more active the better they are mixed with pure sea water. Under these circumstances, their store of energy will be the sooner exhausted" (Gemmill, J. F. 1900, pl. 169). Gemmill was of the opinion, however, that if sperm lived longer in more concentrated suspensions it was because they there received more nourishment, for he goes on to say: "On comparing the movements of spermatozoa in different mixtures, one finds that the difference of activity is not sufficiently marked to account for the very early loss of vitality of spermatozoa in the weaker mixtures simply in terms of exhaustion of energy. I am inclined to believe that the other factor above noted, namely, the dilution of the nutritive medium by the addition of sea water, is the more important cause. An interesting sidelight on this question is afforded by some facts which will be given later regarding the keeping alive of spermatozoa by artificial nutrition" (Gemmill, J. F., 1900, p. 171).[1] An experiment similar to those performed by Gemmill is reported (Table I., Experiment 28). The "term of vitality" of spermatozoa was determined by testing their fertilizing power. As in Gemmill's experiments the sperm in the most concentrated suspensions lived for the longest time.

Table I.

Experiment 28.

The Length of Life, as Measured by the Fertilizing Power, of Sperm Suspensions of Different Concentration.[2]

Date.	Time of Insemination.	Age of Sperm.		1	2	3	4
				Concentration of Sperm Suspensions.			
				4%.	1%.	0.5%.	0.25%.
		Hrs.	Min.	Percentage of Eggs Fertilized when 1 Drop of Sperm Added to 5 Drops of Eggs in 10 c.c. of Sea Water at Intervals as Noted.			
July 25..	12:20 A.M.	0	0				
" " ..	2:30 P.M.	14	10	100	98	67	10
" 26..	12:00 M.	23	40	100	98	15	0
" 27..	11:20 A.M.	47	00	100	0	0	0
" 28..	12:15 P.M.	71	55	98	0		
" 29..	8:20 A.M.	92	00	85			

The measurement of the total carbon dioxide production in sperm suspensions of different concentrations, which will now be reported, has however made untenable the position of Gemmill that the "exhaustion of energy" is not sufficient to account for the "loss of vitality of spermatozoa."

The Measurement of the Total Carbon Dioxide Production of Sperm Suspensions of Different Concentration.

The hydrogen potential of sperm suspensions of varying concentration was measured as a function of time.

In measuring the hydrogen potential of sperm suspensions no such accuracy can be attained as in the measurement of the hydrogen potential of sea water, since the cloudiness of the more concentrated suspensions makes the colorimetric determination difficult and since the intensity of the indicators changes more rapidly in the presence of sperm. This is probably due to the penetration of the indicators into the cells and observations suggest that this depends upon the physiological condition of the sperm. These effects can, however, be prevented from interfering with the measurement of the hydrogen potential of the suspensions if the measurements are made immediately after the indicators are added. If these precautions are observed, a sufficient accuracy for biological purposes can be attained.

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[1] The so-called "artificial nutrition" was brought about by adding beef broth to sea water. This increases the hydrogen ion concentration of the suspension and decreases the activity of spermatozoa. The evidence for these statements will be found in another place in this paper.

[2] The sea water in this experiment was sterilized in order to prevent the bacterial contamination which otherwise occurs when sperm suspensions are kept for so long a time (Gorham, F. R., and Tower, R. W., 1902). Erlenmeyer flasks were used in the experiment. On the third day suspension 1 was still relatively free from bacteria. The spermatozoa appeared healthy and were not motile.

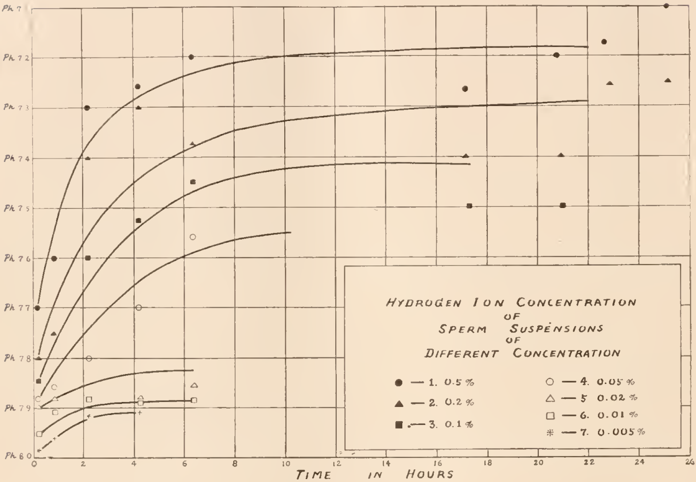

DIAGRAM I.

The data obtained in this way are in Table II. and are graphically represented in Diagram I. The ordinate represents the hydrogen potential of the suspensions; the abscissa, the time in hours. The concentration of sperm in each suspension is recorded in the accompanying tabulation. Since the increase in hydrogen ion concentration is due to the carbon dioxide produced by the spermatozoa, and since the rate of carbon dioxide production is, in turn, a function of the hydrogen ion concentration of the suspension, in all measurable concentrations the carbon dioxide production of sperm suspensions falls off with time.

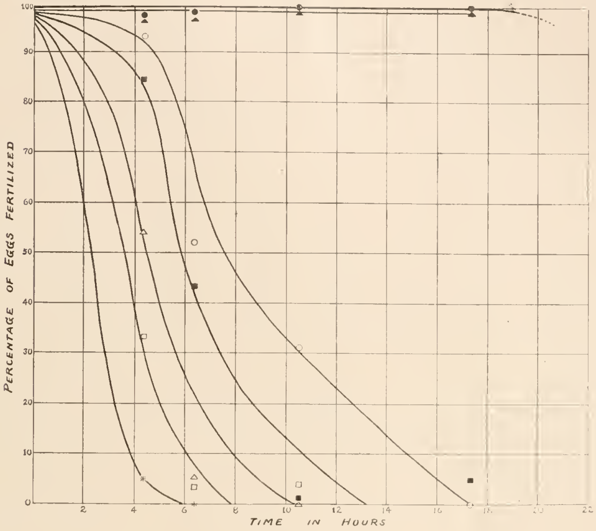

Diagram II.

"The term of vitality of spermatozoa varies according to the degree of their admixture with sea water" (Gemmill, J. F., 1900, p. 170). In the most concentrated suspensions the spermatozoa live for the longest time. The length of life of the different suspensions as computed from the per cent. of eggs that were fertilized by identical concentrations of sperm under identical experimental conditions is recorded in Table III. and graphically represented in Diagram II.

The concentration of carbon dioxide, like the tension of carbon dioxide with which it is in equilibrium, in any one sample of sea water at any one temperature is completely determined by the hydrogen potential. As an approximation, sufficient for the calculation of the carbon dioxide produced by spermatozoa in the above experiment, an increase in carbon dioxide tension (as measured by change in hydrogen potential) may be considered as an increase in free carbonic acid.

TABLE II.

HYDROGEN POTENTIALS OF SPERM SUSPENSIONS.

Age of Sperm Suspensions.		1	2	3	4	5	6	7
		Concentration of the Sperm Suspensions.						
		0.5 %.	0.2 %.	0.1 %.	0.05 %.	0.02 %.	0.01 %.	0.005 %.
Hours.	Minutes	Hydrogen Potentials of Sperm Suspensions.						
	5	7.70	7.80	7.85	7.88	7.90	7.95	7.98
	50	7.60	7.75	7.80	7.86	7.88	7.91	7.96
2	20	7.30	7.40	7.60	7.80		7.88	7.92
4	20	7.26	7.30	7.53	7.70	7.87	7.87	7.91
6	20	7.20	7.37	7.45	7.56	7.86	7.88	7.95
17	20	7.26	7.40	7.50	7.74	7.90	7.98	8.04
20	50	7.20	7.40	7.50				
22	50	7.17	7.26	7.40	(7.74)	(7.88)	(7.91)	(7.98)
24	50	7.10	7.26	7.40				

TABLE III.

LENGTH OF LIFE OF SPERM SUSPENSIONS.

Age of Sperm Suspensions.		1	2	3	4	5	6	7
		Concentration of the Sperm Suspensions.						
		0.5 %.	0.2 %.	0.1 %.	0.05 %.	0.02 %.	0.01 %.	0.005 %.
Hours.	Minutes.	Percentage of Eggs that Were Fertilized in Sea Water by Identical Concentrations of Sperm.						
4	20	98	98	85	94	54	34	6
6	20	99	97	43	52	6	4	0
10	20	100	100	2	31	0	4	7
17	20	100	100	5	0	0	0	0
		Approximate length of life of spermatozoa as computed from their failure longer to fertilize eggs of the same species.						
		17+	17+	17+	10+	6+	6+	4+

The hydrogen potential of sperm suspensions of different concentrations at different times has been reported (Table II.). The total carbon dioxide production of each sperm suspension is equal to the difference between the carbon dioxide concentration of the sea water at the beginning of the experiment and at the time of the death of spermatozoa. If the total carbon dioxide production is divided by the concentration of spermatozoa in the suspension, the carbon dioxide production per unit concentration of sperm is obtained.

The relative carbon dioxide production of sperm suspensions of different concentrations is reported in Table IV.

TABLE IV.

TOTAL CARBON DIOXIDE PRODUCTION OF SPERM SUSPENSIONS.

No.	Sperm Concentration.	Calculation of the Relative Carbon Dioxide Production per Unit Concentration of Sperm.	Approximate Length of Life of Spermatoza.
1	0.5%	13	17+
2	0.2%	18	17+
3	0.1%	26	17+
4	0.05%	37	10+
5	0.02%	32	6+
6	0.01%	45	6+
7	0.005%	60	4+

It is apparent in the most concentrated suspensions that sea water that is more acid than Ph 7.6 destroys spermatozoa, and causes their death before they have been able to expend all their available energy (see p. 192). A post-lethal increase in acidity occurs in such suspensions. McClendon has evidently made similar observations regarding marine invertebrates, for he says: "It would be of little advantage" to determine the carbon dioxide concentrations in sea water more acid than Ph 7.6 "unless it is first absolutely established that the abnormal Ph does not make the organisms physiologically abnormal and that oxygen is still present in the water" (McClendon, Gault, and Mulholland, 1917, p. 33).

In the less concentrated suspensions which more closely simulate normal conditions it will be seen that spermatozoa that live for longer periods of time produce no more carbon dioxide than spermatozoa that live for only 4 hours. In fact the total carbon dioxide production of spermatozoa is an approximate constant. Using the carbon dioxide production as the criterion it must be concluded that the activity of spermatozoa and therefore the life of spermatozoa is limited.

An analysis of the length of life of spermatozoa is essentially, therefore, an analysis of the rate of activity of spermatozoa under varying environmental conditions.

IV. The Effect of the Concentration of the Sperm suspension upon the Activity and upon the Longevity of Spermatozoa.

The observations and the experiments that have been cited show that sea urchin spermatozoa soon become inactive in a certain concentration of hydrogen ions or of carbon dioxide. In lower concentrations spermatozoa are the less active the higher the concentration of hydrogen ions or of carbon dioxide (see also Lillie, F. R., 1913). In the measurements of the carbon dioxide concentration of sperm suspensions that have been reported the sperm produced sufficient carbon dioxide in the more concentrated suspension to inactivate themselves in about two hours. The carbon dioxide production of inactive spermatozoa is of course very much less than the carbon dioxide production of highly active spermatozoa. Since spermatozoa are decreasingly active the higher the concentration of carbon dioxide in the suspension, the observed falling off with time of the carbon dioxide production in all of the suspensions is easily understood.

In very concentrated sperm suspensions, therefore, the spermatozoa are active for but a short time. That undiluted mammalian sperm exhibit but little activity was observed by Koelliker. He remarks: "In der Regel findet sich die Bewegung allerdings nur am Rande des Tropfens, nicht weil hier eine Verdunstung des Samens statt hat, . . . sondern weil am Rande des Tropfens die Intercellular-flüssigkeit in etwas bemerklicherer Weise sich ansammelt" (Koelliker, A., 1856, p. 205). The observation has since been confirmed by many investigators and for nearly all forms. The explanation of the activity of spermatozoa at the border of the drop is contained in the observations of Buller (Buller, A. H., 1902) and Lillie (Lillie, F. R., 1913) quoted in the last section. At the edge of a sperm suspension the oxygen concentration is higher and the carbon dioxide concentration lower than in any other part of the drop. Consequently spermatozoa accumulate in the region of highest carbon dioxide concentration. There they are very inactive, and live for a very long time.

Further evidence that the increased length of life in the more concentrated suspensions is due to the decreased production of carbon dioxide in these suspensions is afforded by the following experiments, which are of two kinds. (1) The length of life of a sperm suspension was either increased by decreasing the rate at which the carbon dioxide produced by the sperm could diffuse from the suspension; or (2) the length of life of the sperm suspension was decreased by decreasing the rate at which the carbon dioxide and hydrogen ion concentration of the suspension increased. This again was attained in two ways. The procedures and the protocols of these experiments will now be reported

V. The Relation between the Length of Life of a Concentrated Sperm Suspension and the Rate at which the Carbon Dioxide produced by Spermatozoa is Eliminated.

1. *Decreasing the Rate of Diffusion of Carbon Dioxide (Experiments 31, 32, and 33).*

In these experiments a sperm suspension was divided between vessels of different diameter. The area of the suspensions that was in contact with the air, and consequently the rate at which the carbon dioxide produced by the sperm escaped into the air constituted the only variable. It was found that the sperm suspensions from which the carbon dioxide could least rapidly diffuse lived for the longest time as judged by the percentage of eggs fertilized when spermatozoa from these vessels were added to eggs in sea water as a function of time (Table V.).

Two methods were employed of decreasing the rate at which the carbon dioxide produced by spermatozoa increases the carbon dioxide and hydrogen ion concentration of the suspension. The procedure in the one (*a*) was essentially the reciprocal of that employed in decreasing the rate of diffusion of carbon dioxide. In the other method (*b*) the "buffer" (Henderson, L. J., 1908) action of sea water and therefore the rate of neutralization of the carbon dioxide was artificially increased.

(2*a*) *Increasing the Rate of Diffusion of Carbon Dioxide.*

In experiment 214 the sperm suspension was divided between two shallow vessels. The one remained in contact with the air of the room, while over the surface of the other a stream of carbon

TABLE V.

THE RELATION BETWEEN THE LENGTH OF LIFE OF A CONCENTRATED SPERM SUSPENSION AND THE RATE AT WHICH THE CARBON DIOXIDE PRODUCED BY SPERMATOZOA DIFFUSES FROM THE SUSPENSION.

Experiment 32.

Concentration of Suspension.	Time of Insemination.	Age of Sperm.		A.	B.	C.	D.	No. of Drops of Sperm Added to Eggs in Sea Water.
				Approximate Area in Square Centimeters of the Interface Between Air and Sperm Suspension.				
				3.	20.	80.	500.	
		Hrs.	Min.	Percentage of Eggs Fertilized when Sperm Added to Four Drops Eggs in 5 c.c. of Sea Water.				
0.04%	8.10	0	0	86	62	59		1
	9.30	1	20	19	10	5		
	10.10	2	00	3	1	0		
0.04%	8.10	0	0					4
	9.30	1	20	41	13	11		
	10.10	2	00	31	3	3		
	10.25	2	15	11	0	0		
Experiment 33.								
0.05%	12.30	0	0					1
	12.35	0	5	94		98	67	
	1.20	0	50	57		43	6	
0.05%	1.20	0	50	99		94	54	8
	2.40	2	10	69		28	7	
	3.35	3	5	34		15	4	
	4.45	4	15	16		41	3	
Experiment 31.								
0.04%	11.07	0	0	99	100			1
	12.07	1	00	100	60			
	12.37	1	30	96	21			
	12.57	1	50	99	23			
	1.30	2	23	90	14			
	2.00	2	53	61	7			
0.04%	11.07	0	0	100	100			8
	12.07	1	00	100	100			
	12.37	1	30	100	100			
	12.57	1	50	100	64			
	1.30	2	23	100	33			
	2.00	2	53	100	35			

dioxide free air was continuously drawn. In this way the carbon dioxide concentration of the one suspension was prevented from increasing at the same rate as that of the other. As a result the carbon dioxide production of the spermatozoa in the latter case was not so much inhibited. The life of the sperm suspension was, therefore, shortened.

TABLE VI.

THE RELATION BETWEEN THE LENGTH OF LIFE OF A CONCENTRATED SPERM SUSPENSION AND THE RATE AT WHICH THE CARBON DIOXIDE PRODUCED BY SPERMATOZOA DIFFUSES FROM THE SUSPENSION.

Experiment 214.

Concentration of Suspension.	Time of Insemination.	Age of Sperm.		*A.*	*B.*
				Approximate Carbon Dioxide Tension of the Air with which the Surface of the Suspension Was in Contact.	
				About 0.3 Mm.	About 0.1 Mm.
		Hrs.	Min.	Percentage of Eggs Fertilized when One Drop of Sperm is Added to Ten Drops Eggs in 10 c.c. of Sea Water.	
0.02%	12:00 M.	0	0		
	12.15 P.M.	0	15	100[1]	100[1]
	1.30	1	30	100[1]	100[1]
	3.30	3	30	100[1]	100[1]
	5.30	5	30	100[1]	100[1]
	7.15	7	15	100[1]	100[1]
	9.35	9	35	100[1]	85
	12.35 A.M.	13	35	100[1]	77
	1.35	13	35	100[1]	74
	10.30	22	30	90	19
	12.15 P.M.	24	15	89	20
	1.30	25	30	92	32
	5.00	29	00	93	25
	8.00	32	00	81	1
	11.50	35	50	20	0

(2*b*) *Increasing the Rate of Neutralization of Carbon Dioxide.*

When a mixture of a weak acid and its salt, isohydric with sea water, is added to sea water, a series of solutions is obtained that tend increasingly to maintain the reaction of the sea water. The carbon dioxide produced by spermatozoa or other organisms changes the hydrogen ion and the carbon dioxide concentration of such solutions the less, the greater the concentration of weak acids.

In the experiments to be reported mixtures of borax and boric acid were used (Palitzsch, 1914). It is, of course, necessary that neither the weak acid nor the salts of the weak acid that are formed with the ions of sea water be toxic to the organism under investigation. According to a personal communication, C. M. Child has observed distinct toxic action of the concentrations of borates used in these experiments upon developing sea urchin eggs. It is therefore possible that the action of borates in shortening the life of the sperm is in part due to their toxicity.

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[1] Fertilization was seen to be practically complete and the percentage of eggs cleaving was only estimated.

TABLE VII.

THE RELATION BETWEEN THE LENGTH OF LIFE OF A CONCENTRATED SPERM SUSPENSION AND THE RATE AT WHICH THE CARBON DIOXIDE PRODUCED BY SPERMATOZOA IS NEUTRALIZED BY ISOHYDRIC BORATES.

Experiment 208.

Concentration of Suspension.	Time of Insemination.	Age of Sperm.		*A.*	*B.*	*C.*	*D.*	*E.*	*F.*	Ph of Borate Mixture.
				No. of c.c. of Borate Mixture Added to 10 c.c. of the Sea Water in the Sperm Suspensions.						
				0.	0.1.	0.3.	0.5.	1.0.	2.0.	
		Hrs.	Min.	Percentage of Eggs Fertilized when One Drop of Sperm Added to Five Drops Eggs in 5 c.c. of Sea Water.						
0.1%	2.25 P.M.	0	0	100	100	100	100	99	100	8.41
	2.55	0	30	100	97	98	26	47	8	
	3.25	1	00	92	82	57	55	10	1	
	4.00	1	35	52	21	14	13	0	0	
Experiment 205.										
0.2%	11.20 A.M.	0	0	100	100	100	100	100		8.41
	11.50	0	30	100	100	100	99	100		
	1.40 P.M.	2	20	66	28	40	4	2		
Experiment 209.										
1.0%	10.00 A.M.	0	0	100					50	8.41
	5.20 P.M.	7	20	98	25	6	1	3	0	
Experiment 223.										
1.0%	4.00 P.M.	0	0							8.08
	10.00 A.M.	18	0	88	80	77	70			
	11.30 A.M.	19	30	85	86	27	87			
	[1] 2.00 P.M.	22	00	100	100	28	19			
1.0%	4.00 P.M.	0	0							8.51
	10.00 A.M.	18	0	88	82	3	8			
	11.30	19	30	85	80	2	2			
	2.00 P.M.	22	00	100	100	9	27			

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[1] The eggs of a female that had been opened at 1.30 P.M. were used in this series of inseminations. They were evidently more easily fertilized than the eggs of the female that had been opened at 9.30 A.M.

In suspensions with the highest concentration of weak acids the spermatozoa lived for the shortest time. For the higher the concentration of weak acids the smaller the change in the hydrogen ion and carbon dioxide concentration of the suspensions due to the carbon dioxide produced by the spermatozoa. Consequently spermatozoa in more alkaline solutions, where they were most active, lived for the shortest time. In order the better to prevent spermatozoa from being inactive, a borate mixture that was slightly more alkaline than sea water was employed in the first three experiments reported (Experiments 205, 208, 209). The hydrogen potential of the borate mixture is recorded in the last column of Table VII.

That even this slight change in the hydrogen potential of the borate effects spermatozoa is demonstrated in Experiment 223 where slightly different mixtures of borates were used in the same concentration. It will be seen that spermatozoa in the suspension to which had been added the more alkaline mixture, lived the shorter time.

VI. The Effect of the Hydrogen Ion Concentration of the Suspension upon the Activity and upon the Longevity of Spermatozoa.

The activity of spermatozoa is a function of the hydrogen ion concentration. Repeated observation has confirmed this relation. Since there is a definite relation between the activity and the length of life of spermatozoa, the latter is also a function of the hydrogen ion concentration. The hydrogen ion concentration of the ocean at Woods Hole, Massachusetts, is about 0.1×10^{-7} (Ph 7.95 to Ph 8.15). The weak acids (Henderson, L. J., and Cohn, E. J., 1916) and the currents prevent the hydrogen ion concentration of the ocean from appreciably changing. In such a solution the length of life of spermatozoa is short.

Loeb has observed the simultaneous spawning of the sea urchins (*Strongylocentrotus purpuratus*) at the shore of Pacific Grove. "At such spawning seasons the sea water becomes a suspension of sperm" (Loeb, J., 1916, p. 94). It would be interesting to know whether the hydrogen ion concentration of such a suspension increases.

Such concentrations of spermatozoa as were used in the experiments reported in the last section probably never occur in the ocean. The conditions that obtained in these experiments approximate those of ripe sperm in the testes very much more closely than they do those of sperm that are shed into the ocean. The hydrogen ion concentration in these suspensions increased not inconsiderably as a result of carbon dioxide produced by the sperm. This rise in carbon dioxide (and also in hydrogen ion) concentration was measured. The hydrogen ion and carbon dioxide concentration of the suspensions in which spermatozoa lived for the longest time was sufficiently great to inactivate spermatozoa. The length of life of concentrated sperm suspensions is therefore for the most part ascribable to the hydrogen ion concentration of such suspensions.

The length of life of a sperm suspension at different hydrogen ion concentrations was determined.

Two criteria of the length of life of the sperm were employed. The fertilization tests are reported. The hydrogen potential of the sea water was determined by colorimetric comparison with standardized mixtures of borates or phosphates in the way that that has already been described (p. 171).

In the experiments to be reported the hydrogen ion concentration was decreased by the addition of sodium hydroxide to sea water. The hydrogen ion concentration was increased by the addition of hydrochloric acid.

When an acid stronger than carbonic acid is added to sea water, carbonic acid is displaced from its salts and carbon dioxide is set free. As the carbon dioxide that is set free diffuses from the solution the hydrogen ion concentration decreases, until the carbon dioxide of the sea water is again in equilibrium with the partial pressure of that gas in the air. The hydrogen ion concentration that is eventually reached is different from the original hydrogen ion concentration of the sea water, but nearer to it than to the hydrogen ion concentration immediately after the acid is added. This regulation of the neutrality persists until all of the carbonates have been decomposed. The rate at which equilibrium is approached depends upon the temperature, the surface, and the degree of agitation.

Because of this property of carbonate solutions it is necessary to know not only the hydrogen ion concentration but also the carbon dioxide tension. The latter is, as stated, above expressed by the number of millimeters of mercury that represents the partial pressure of the gas. The procedure usually followed was to restore the equilbrium between the sea water (of whatever hydrogen ion concentration) and the carbon dioxide of the air, before beginning an experiment. This was accomplished either by shaking with air, or by bubbling air through the solutions.

In a series of experiments it was found that the more alkaline the solution (*i. e.*, the lower the hydrogen ion concentration) the shorter the life of the sperm. The activity of the spermatozoa is increased in these suspensions, and spermatozoa that are added to ripe eggs in sea water while in this activated condition have a greater "fertilizing power" (this has been previously reported. See Fuchs, H. M., 1915) than spermatozoa that have been in less alkaline sea water. This lasts for a much shorter time, since the life of spermatozoa is very short in alkaline solution. (If the hydrogen potential is greater than about Ph 9.4 spermatozoa are instantly agglutinated.) In order to demonstrate the increased "fertilizing power" of spermatozoa that have been in alkaline solution it is necessary to inseminate in such dilution that the spermatozoa that have been in sea water with greater hydrogen ion concentration do not fertilize all of the eggs.

Experiment 227 illustrates both the effect of alkaline sea water in increasing the "fertilizing power" for a short time and of more acid sea water in increasing the time during which the "fertilizing power" is exhibited. The reversal in the effect of alkaline sea water upon the "fertilizing power" of spermatozoa was demonstrable only because the eggs used in the first part of this experiment were fertilized with difficulty. Otherwise the early fertilizations would have been complete, and the effect of alkaline sea water upon spermatozoa not have been observed. Diagram III. represents the prolongation of the life of the sperm in acid suspension. The ordinates measure the fertilizing power of the sperm at the times designated by the abscissæ. The hydrogen potentials of the suspensions are symbolically recorded.

If sea water is much more alkaline than Ph 9.4, spermatozoa are instantly agglutinated. If sea water is about Ph 7.6, spermatozoa are inactive. Sea water that is much more acid than Ph 7.6 not only tends to inactivate, but also to destroy spermatozoa and the more so the higher the hydrogen ion concentration and the longer the sperm are subjected to these acidities.

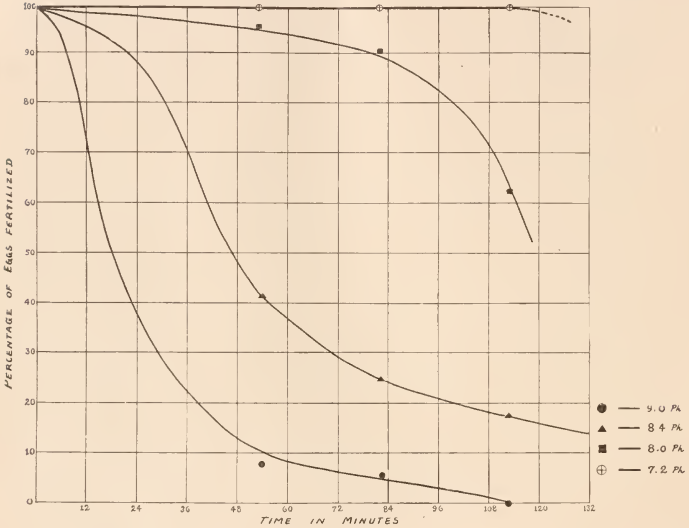

DIAGRAM III.

In sea water that is less acid than Ph 7.6 spermatozoa live the longer the higher the hydrogen ion concentration. The more inactive the spermatozoa the more slowly their "fertilizing power" diminishes when they are transferred to sea water where they are reactivated.

VII. THE RELATION BETWEEN THE ACTIVITY AND THE "FERTILIZING POWER" OF SPERMATOZOA.

"Within a wide limit of egg-concentration the important factors in fertilizing power of sperm suspensions are: (1) concentration, (2) time" (Lillie, F. R., 1915, p. 246). The results of this investigation confirm this general conclusion of Lillie's, and add one more factor, the hydrogen ion concentration. For the length of life and the "fertilizing power" of a sperm suspension are dependent on the hydrogen ion concentration of the suspension, and the "fertilizing power" of spermatozoa at the same hydrogen ion concentration is in some way dependent upon the sperm concentration.

The Effect of the Hydrogen Ion Concentration upon the Fertilizing Power of Spermatozoa.

The loss of "fertilizing power" of active sperm suspensions of approximately the same concentration (0.04 per cent.) at different hydrogen ion concentrations is graphically represented in Diagram IV. The curves are plotted from the data in Table VIII. on the basis of maximum "fertilizing power" at Ph 9.0. If but 50 per cent. of the eggs were fertilized at Ph 9.0 (as in experiment 227–I.) and 24 per cent. at Ph 8.4 the ratio of the "fertilizing powers" is of course as 100 to 48.

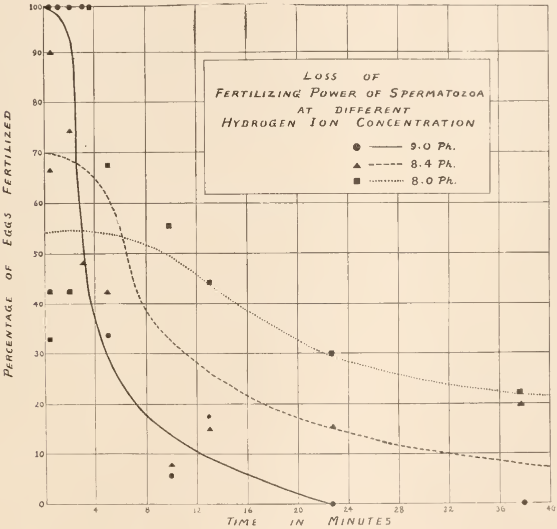

Diagram IV.

Table VIII.

The Effect of the Hydrogen Ion Concentration of the Suspension upon the Fertilizing Power and upon the Length of Life of Spermatozoa.

Concentration of Suspension.	Time of Insemination.	Age of Sperm.		C.c. of 0.02N HCl to 10 c.c. Sea Water.				C.c. of 0.2N NaOH to 10 c.c. Sea Water.				No. Drops of Sperm Added to Eggs.
				1.1.	1.0.	0.5.	0.	.06.	.12.	.25.	.50.	
				Hydrogen Potential of Sperm Suspensions at CO_2 Tension of Air.								
						7.7.	8.0.	8.2.	8.4	8.6.	9.0.	
		Hrs.	Min.	Percentage of Eggs Fertilized when Sperm is Added to Five Drops Eggs in 5 c.c. of Sea Water.								
Experiment 20.												
0.015%	11.51		0									1
	11.53		2				41	48	67	51	98	
	1.00	1	9				0	0	0	0	0	
Experiment 226.												
0.04%	4.40		0									1
	4.42		2				20	39	35	47	47	
	4.45		5				32	18	20	18	16	
	4.50		10				26	32	4	34	3	
Experiment 21.												
0.045%	12.25		0				33	94	90	90	99	4
	1.25	1	0				5	36	23	1	3	
	2.20	1	55				1	7	7	0	1	
Experiment 227—I.												
0.08%	11.07		0									1
	11.10		3	4	38	7	51		24		50[1]	
	11.20		13	11	60	15	22		8		9	
	11.30		23	19	31	18	15		8		0	
	11.45		38	13	44	0	11		10		0	
Experiment 227—II.[2]												
	12.00		53	100	100	89	96		41		8	
	12.30	1	23	100	100	67	90		25		6	
	1.00	1	53	100	100	60	63		17		0	
Experiment 22.												
0.7%	12.25		0									1
	12.40		15				100	100	100	100	100	
	1.15		50				98	100	100	100	100	
	5.20	4	55				35	86	90	5	0	

[1] But 0.3 c.c. of sodium hydroxide had been added in Expt. 227. The Ph was 8.8.

[2] Same sperm used as in 227—I. The eggs, however, of another female.

Spermatozoa that have been in alkaline sea water have a greater "fertilizing power" than spermatozoa that have been in sea water at the hydrogen ion concentration of the ocean. This becomes apparent only at dilutions such that the spermatozoa that have been in the less alkaline sea water do not fertilize all of the eggs. If all of the eggs are not fertilized, it must be either (*a*) because there are not enough spermatozoa or (*b*) because the spermatozoa are not sufficiently potent. Since the same concentration of sperm is able to fertilize completely at another hydrogen ion concentration, the first alternative does not explain the results in these experiments (Experiment 20, 21, 226, 227–I.).

If the "fertilizing power" of active spermatozoa were exactly proportional to the energy expended, the areas beneath the curves in the diagram would be exactly equal, since the total available energy of spermatozoa is practically constant (p. 183). If the relation is not as simple as this, it is at all events perfectly definite and definable, and strongly suggestive of a simple functional relation, at least during the first few minutes of the life of the sperm.[1]

The Effect of the Sperm Concentration upon the "Fertilizing Power" of Spermatozoa.

"In his epoch-making 'Expériences pour servir a l'histoire de la génération des animaux et des plantes' published in 1785 the Abbe Spallanzani describes among his numerous experiments on fertilization and artificial parthenogenesis some determinations concerning the minimal quantity of sperm necessary to fertilize the eggs of the frog" (Lillie, F. R., 1915, p. 229). "In 1824 Prevost et Dumas confirmed these" results (Lillie, F. R., 1915, p. 229). More recently in the "Analysis of Variations in the Fertilizing Power of Sperm Suspensions of *Arbacia*" (Lillie, F. R., 1915, p. 229) that has already been quoted, F. R. Lillie demonstrated that at "a dilution of 1/10,000 per cent.," where "one can rarely find a single spermatozoön in the jelly of the fertilized

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[1] Thereafter it is difficult to conceive of the physiological condition of the spermatozoön as suffering no alteration. A change in the physiological condition must in turn affect the "fertilizing power." Strong evidence for the view that the ageing of Echinid sperm affects its physiological condition has previously been presented by Dungay (1913) and Vernon (1899).

TABLE IX.

THE EFFECT OF THE HYDROGEN ION CONCENTRATION OF THE SUSPENSION UPON THE FERTILIZING POWER AND UPON THE LENGTH OF LIFE OF SPERMATOZOA.

Experiment 228.

Concentration of Suspension.	Time of Insemination.	Age of Sperm.		C.c. of .02N HCl to 10 c.c. Sea Water.					No Drops of Sperm Added to Eggs.
				1.0.	0.9.	0.8.	0.5.	0.0.	
				Hydrogen Potential at CO_2 Tension of Air.					
						7.4.	7.7.	8.0.	
		Hrs.	Min.	Percentage of Eggs Fertilized when Sperm is Added to Five Drops Eggs in 5 c.c. of Sea Water.					
0.001%	11.55		0						1
	12.00		5		6	12	31	19	
	12.15		20		1	0	4	2	
	1.00		65		0	0	0	0	
0.005%	11.55		0						1
	12.00		5		41	71	72	86	
	12.15		20		1	0	3	29	
	1.00		65		0	0	1	0	
0.01%	11.55		0						1
	12.00		5		71?	31	94	100	
	12.15		20		7	1	78	86	
	1.00		65		0	0	33	5	
Experiment 215.									
0.25%	12.05		0						1
	12.30		25	84		78	86	84	
	9.00	8	55	12		66	87	6	
	12.40	12	35	0		7	23	0	

eggs" (Lillie, F. R., 1915, p. 234) about 95 per cent. of the eggs were fertilized if the time, which "is an extremely important factor with reference to fertilizing power" (Lillie, F. R., 1915, p. 234) during which the sperm were in sea water, was short. For: "If the sperm suspensions lose their fertilizing power with time, it must be that the significance of time in this respect varies inversely to concentration" (Lillie, F. R., 1915, p. 239).

I believe that Lillie's results have conclusively demonstrated that one sperm is quite sufficient to fertilize an egg if it has not already expended a large part of its available energy. Thereafter the statement that "the initiation of development by a single spermatozoön . . . is impossible because a single sperm cannot affect those changes in the egg-coverings" (Glaser, O., 1915, p. 153) which are necessary to fertilization, has unquestioned significance. The older the spermatozoa and the more their available energy has been expended, the more spermatozoa are necessary in order to effect fertilization. There is, however, even then no doubt that "a single spermatozoön is sufficient to carry out the bi-parental effect" (Glaser, O., 1915, p. 153).

The "fertilizing power" of sperm at the same hydrogen ion concentration is, therefore, in some way dependent on the concentration of the sperm. In Experiment 228 in Table IX. (p. 196) a 0.001 per cent.; a 0.005 per cent. and a 0.01 per cent. suspension was made from the sperm of one male. The "fertilizing power" at each hydrogen ion concentration was lost the sooner the smaller the concentration of sperm.

It is, of course, true that in all but the most dilute suspensions the hydrogen ion concentration will in a short space of time be the higher the more concentrated the suspension as a result of the carbon dioxide produced by the sperm. This probably occurred in Experiment 22, but it is improbable that this was the only factor in the other experiments, and it certainly was not a factor in the data that will now be presented.

In Experiments 31, 32 and 33 (p. 186) different amounts of sperm from the same suspension were added to the same concentration of eggs. These experiments are retabulated in order that the percentage of eggs that were fertilized by different amounts of sperm from the same suspension at the same time can more easily be compared. When all of the eggs were not fertilized by both concentrations of sperm the number of eggs fertilized was always the greater the more spermatozoa were added.[1]

This is not a new observation. It is the common experience of investigators, and may be explained either by assuming that in the greater concentration more sperm will on the average arrive at the periphery of the egg with sufficient action (having the dimensions of energy × time) to initiate development, or by assuming that mass action of spermatozoa may be effective in initiating the development of the egg. This implies that what is true in parthenogenesis is also true in normal fertilization. R. S. Lillie (Lillie, R. S., 1916) has quite beautifully shown that the product of the concentration of the parthenogenetic agent and the length of time during which it is necessary to subject eggs to the agent in order to initiate development is a constant. The evidence that has been presented strongly suggests a similar quantitative relation in fertilization.

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[1] It should be pointed out that whereas motility is unquestionably a manifestation of energy, the observation of motile spermatozoa at the periphery of an unfertilized egg in no way indicates the physiological condition of the spermatozoa.

TABLE X.

THE RELATION BETWEEN THE CONCENTRATION OF THE SPERM AND THE PERCENTAGE OF EGGS THAT ARE FERTILIZED.

Number of Experiment.	Designation of Suspension.	Age of Sperm Suspension.		Amount of Sperm Added.		Amount of Sperm Added.	
		Hrs.	Min.	1 Drop.	4 Drops.	1 Drop.	8 Drops.
				Percentage of Eggs that are Fertilized.		Percentage of Eggs that are Fertilized.	
32	*A*	1	20	19	41		
	B	1	20	10	13		
	C	1	20	5	11		
	A	2	0	3	31		
	B	2	0	1	3		
	C	2	0	0	3		
33	*A*		50			57	99
	B		50			43	94
	D		50			6	54
31	*A*	1	0			100	100
	B	1	0			60	100
	A	1	30			96	100
	B	1	30			21	100
	A	1	50			99	100
	B	1	50			23	64
	A	2	23			90	100
	B	2	23			14	33
	A	2	53			61	100
	B	2	53			7	35

VIII. THE EFFECT OF THE CARBON DIOXIDE CONCENTRATION UPON THE ACTIVITY AND UPON THE LONGEVITY OF SPERMATOZOA.

The effect of increasing the carbon dioxide concentration of the suspension is to increase the length of life of the spermatozoa.

TABLE XI.

THE EFFECT OF THE CARBON DIOXIDE TENSION UPON THE LENGTH OF LIFE OF SPERMATOZOA.

Experiment 225.

Concentration of Suspension.	Time of Insemination.	Age of Sperm.		CO_2 Tensions of the Suspensions in Mm.					No. Drops Sperm Added to Eggs.
				16.	4.	2.	1.	0.3+.	
				Hydrogen Potentials of the Suspensions.					
				6.5.	7.0.	7.3.	7.6.	8.0.	
		Hrs.	Min.	Percentage of Eggs Fertilized when Sperm Added to Eggs in 5 C.C. of Sea Water.					
0.04%	4.40	0	0						[1]
	9.00	4	20	10	7		97	53	
	11.45	7	5	0	4		41	16	
Experiment 212.									
0.2%	12.05	0	0						1
	12.10		5	100	100		100	100	
	12.25		20	100	100		100	100	
	2.30	2	25	100	100		100	100	
	4.40	4	35	99	100		100	100	
	11.50	11	45	100	100		100	100	
	9.55	21	50	7	4		94	80	
	12.20	24	15	1	0		100	100	
	4.50	28	45	2	15		92	90	
Experiment 213.									
0.2%	12.10	0	0						1
	12.20	0	10			100		100	
	1.50	1	40			100		55	
	3.30	3	20			98		96	
	4.50	4	40			100		99	
	9.40	9	30			99		95	
	9.40	21	30			100		20	
	12.15	24	5			84		5	

For as the carbon dioxide concentration of sea water increases, so also does the concentration of carbonic acid and of hydrogen ions. In several experiments the length of life of a sperm suspension at different carbon dioxide tensions[2] was determined and sperm were found to live for the longest time when the carbon dioxide tension was about one millimeter. The hydrogen potential of sea water at a carbon dioxide tension of one millimeter is about 7.6 (Henderson, L. J., and Cohn, E. J., 1916). But this is the hydrogen potential that has been found to be most effective in increasing the length of life of spermatozoa. Three experiments are reported in Table XI. It will be seen that when the carbon dioxide tension is greater than one millimeter and the hydrogen potential less than 7.6, sperm are destroyed. This is in complete agreement with what has already been found with respect to the hydrogen ion concentration.

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[1] In this experiment five drops of eggs were added to the undiluted suspensions.

[2] The relation between the tension and the concentration of carbon dioxide is discussed on page 172.

It is not maintained that the only effect of carbon dioxide upon the physiological condition of spermatozoa is brought about by ionized hydrogen. On the contrary, several experiments, which are unfortunately not conclusive (and are therefore not reported) indicate a difference in the subsequent behavior of spermatozoa that are subjected to the same hydrogen ion concentration but to different carbon dioxide tensions. The fact that the total carbon dioxide production per unit concentration of sperm in concentrated suspensions is less than in more dilute suspensions (Table IV.) suggests that the rate at which carbon dioxide is eliminated depends in some way upon the difference in carbon dioxide tension between the spermatozoon and its environment.

IX. The Effect of the Oxygen Concentration upon the Activity and upon the Longevity of Spermatozoa.

The generalization may be hazarded that whatever decreases the activity increases the length of life of spermatozoa and conversely that whatever increases the activity decreases the length of their life. Buller (Buller, A. H., 1902) observed the differential activity of spermatozoa in an oxygen gradient (see p. 175). Drzewina and Bohn (Drzewina, A., and Bohn, G., 1912) have demonstrated that sperm live for a long time in an oxygen-poor medium.

Drzewina and Bohn have also demonstrated that the addition of KCN to sea water prolongs the life of the sperm. Loeb (1915) has shown that spermatozoa are immobilized by NaCN, and it is certain that the length of their life is thereby increased. "It is a well-known fact that the unfertilized eggs of the sea urchin (in fact of all marine animals) perish when they lie for some time in sea water and one of the main causes of this phenomenon is also known, namely oxidations. If the oxidations are inhibited through the removal of oxygen or the addition of KCN the life of the eggs can be prolonged"[1] (Loeb, J., 1915, p. 282).

It is probable that oxygen lack also plays a part in increasing the length of life of concentrated sperm suspensions. McClendon (McClendon, Gault and Mulholland, 1917) has recently estimated that respiration that raises the hydrogen potential of sea water (of excess base 23) to approximately 7.6 uses up all of the available oxygen.

The experiments of Drzewina and Bohn have been repeated and the results substantially confirmed. Spermatozoa are quite inactive in the concentrations of KCN that are most effective in prolonging the life of spermatozoa. The results of several experiments follow.

TABLE XII.

THE EFFECT OF THE ADDITION OF KCN TO SEA WATER ON THE LENGTH OF LIFE OF THE SPERM SUSPENSION.

Experiment 14.

Concentration of Suspension.	Time of Insemination.	Age of Sperm.		Number of C.C. of $0.1N$ KCN Added to a Liter of Sea Water.								No. of Drops Sperm Added to Eggs.
				10	2.5	1.25	0.62	0.31	0.16	0.04	0	
		Hrs.	Min.	Percentage of Eggs Fertilized when Sperm Added to Eggs in 5 C.C. Sea Water.								
0.04%	11.05	0	0	99	97		97		93	97	71	8
	12.05	1	0	100	98						91	
	1.40	2	35	1	100						34	
	2.45	3	40	2	100		79		16	26	4	
	7.15	8	10		73							

Experiment 27.

0.7%	3.45	0	0									1[2]
	4.40	0	55		100	100	100	100			100	
	9.50	18	5		100	100	100	100			100	
	9.45	42	0		100	5	99	98			97	
	2.00	46	15		98	0	4	2			2	
	4.45	49	0		97		6	0			0	
	7.00	51	15		2		1					

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[1] The way in which KCN effects the oxidations of cells need not be discussed in connection with these experiments. For a discussion of this question see Lillie, R. S., 1916, page 311, and Child, C. M., 1915, p. 66).

[2] Insemination took place in 10 c.c. of sea water in this experiment.

X. The Effect of Beef Broth upon the Activity and upon the Longevity of Spermatozoa.

Gemmill showed that the length of life of spermatozoa could be increased by adding beef broth to sea water. He believed that this brought about the "keeping alive of spermatozoa by artificial nutrition" (Gemmill, J. F., 1900, p. 171) (see p. 178).

The work of Gemmill has been repeated and his observation that sea water to which beef broth has been added in appropriate concentration prolongs the life of spermatozoa, confirmed. The activity of spermatozoa, however, is decreased in the beef broth suspensions. Moreover the addition of beef broth to sea water increases the hydrogen ion concentration.[1]

It is unfortunately not possible to neutralize the suspension without throwing down a heavy precipitate.[2] This brings about such changes in the ionic composition of the sea water as to make uninterpretable the results of experiments carried out in such a medium.

The protocols of two experiments that confirm the results of Gemmill are recorded.

2.5 grams of Armour's "extract of beef" were dissolved in 290 c.c. of sea water, making the concentration by weight approximately 8.6 per cent. The suspension of beef broth in sea water was acid to neutral red. That is, the hydrogen ion concentration was greater than Ph 6.5. Diluting the suspension at once decreased its concentration in beef broth and in hydrogen ions. The hydrogen ion concentrations were not directly determined, but their approximate value has been calculated from the number of cubic centimeters of 0.01 N NaOH required to make them alkaline to phenolphthalein.

To 10 c.c. of the different concentrations of acid broth one drop of dry sperm was added. Tests were made by adding one drop of each sperm suspension to five drops of eggs in 10 c.c. of sea water. The percentage of eggs that were fertilized are reported in Table XIII.

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[1] The increase in viscosity of the suspension, and also the increase in protein content, may be effective in decreasing the activity of spermatozoa.

[2] In all probability an insoluble calcium salt or aggregate.

Table XIII.

The Effect of the Addition of Beef Broth to Sea Water upon the Length of Life of Spermatozoa.

Experiment 35.

Concentration of Suspension.	Time of Insemination.	Age of Sperm.		Concentration of Beef Broth in Sea Water.						No. of Drops Sperm Added to Eggs.
				8.6%.	4.3%.	2.1%.	1.1%.	0.5%.	0.0%.	
				Probable Hydrogen Potential of the Suspensions.						
				6.5.	7.2.	7.6.	7.8.	7.9.	8.0.	
		Hrs.	Min.	Percentage of Eggs Fertilized when Sperm Added to Five Drops Eggs in 10 C.C. of Sea Water.						
0.02%	11.55	0	0	61	81	99	99		93	1
	12.25	0	30	2	92	100	58		0	
	12.48	0	53	0	61	88	99		2	
	1.15	1	20	0	5	79	67		0	
	1.45	1	50	0	0	2	46		0	
Experiment 34.										
0.7%	10.05	18	35	0	98	99	98	99	97	1
	2.45	23	15	0	94	97	95	98	96	
	6.20	26	50	0	53	95	94	79	30	
	10.10	30	40	0	32	89	98	43	20	

XI. The Effect of "Egg Water" upon the Activity and upon the Longevity of Spermatozoa.

Owing to the fact that sperm are "agglutinated" by the water in which eggs of the same or closely related species have been allowed to stand, much interest has centered around the effects of the so called "egg water" or "fertilizin" upon the fertilizing power of spermatozoa.

A. H. Buller seems to have been one of the first students of the fertilization process in Echinids to have noticed this phenomenon. In 1900 he reported before the British Association: "In the case of *Arbacia* it was discovered that when spermatozoa are introduced into a drop containing freshly extruded eggs they collect into small balls, often composed of 100 or more individuals. The balls were also formed after the water had received four successive filtrations. A tactile stimulus appears to play a part in the phenomenon" (Buller, A. H., 1900, p. 387).

Since then this phenomenon and the properties of the agglutinating "egg water"[1] have been successively studied by E. von Dungern (1901) and (1902); A. Schücking (1903) J. De Meyer (1911); F. R. Lillie (1912, 1913, 1914, 1915); H. M. Fuchs (1915); Jacques Loeb (1914, 1915); Otto Glaser (1913, 1914); A. Richards and A. E. Woodward (1915) and A. E. Woodward (1915). Each investigator has conceived the function of the "egg water" and its importance in the fertilization process to be different. It is not the purpose of the present communication to consider the function of the "egg water" (although that is a problem of great biological interest) but the behavior of the spermatozoön, and it has been possible to repeat and to explain many of the seemingly contradictory observations of different investigators on the effect of "egg water" upon the fertilizing power and upon the length of life of spermatozoa.

The effect of "egg water" upon spermatozoa as was clearly shown in the admirable investigation of Schücking depends upon the relative concentration of egg water and sperm; upon the absolute concentration of each; and upon the length of time during which sperm are allowed to remain in the egg water. Schücking observed that: "Die sauer reagirende Eimasse übt bei den genannten Echinodermen eine tödtliche, bei kurzer Dauer der Einwirkung lähmende, in geringer Menge agglutinirende bezw. erregende und anlockende Wirkung auf Spermien der eigenen und fremden Art aus" (Schücking, A., 1903, p. 91).

In a more complete analysis of the phenomenon of activation and agglutination F. R. Lillie (1913) showed that if "egg water" is added to a sperm suspension the activity of the spermatozoa is greatly increased. One of the manifestations of this increased activity is the "agglutination" phenomenon. According to Gray "if a drop or two of a very weak solution of cerous chloride is added to a suspension of *Arbacia* sperm in sea water the spermatozoa become intensely active, and rapidly aggregate into clumps" (Gray, J., 1915, p. 123). This may possibly be (Lillie, F. R., 1915, p. 20) what Lillie now calls "mass coagulation," which was described by Loeb in 1904 (Loeb, J., 1904) and is favored not only by increase in the hydroxyl ion concentration but by increase in the concentration of bivalent ions, notably calcium (Loeb, J., 1914). Unlike "agglutination" it is not reversible. In an earlier paper Lillie did not distinguish between the two phenomenon, for he wrote: "Agglutination is not in itself a specific process; it may take place spontaneously to a certain extent under some conditions; it is caused by increase of alkalinity of the sea-water" (Lillie, F. R., 1913, p. 563). Loeb has designated Lillie's "agglutination" as "cluster formation."

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[1] The distilled water "extract" of Echinid eggs has been found to possess many of the properties of the "egg water."

It will be remembered that increasing the alkalinity also increases the activity of spermatozoa. Loeb (1914) has shown that inactive sperm do not exhibit the reversible "agglutination."[1] The irreversible agglutination, or "mass coagulation" is independent of the motility of the spermatozoa.

If the "egg water" is of sufficient strength,[2] however, the sperm are completely non-motile after the initial period of activation. By adding eggs to such spermatozoa Lillie showed that their fertilizing power was slight. "The powerful effect of the egg extract on spermatozoa of the same species may be shown by a complete loss of motility as we have already seen, and also by a corresponding loss or diminution of the fertilizing power" (Lillie, F. R., 1913, p. 558).

Fuchs (Fuchs, H. M., 1915) in experiments in which sperm that had been treated with "egg water" were added to eggs in sea-water showed that the fertilizing power of the sperm had been increased by the "egg water."[3]

But an analysis of the effect of "egg water" upon the fertilizing power of spermatozoa must differentiate between the following effects of "egg water" upon the physiological condition of spermatozoa.

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[1] That "agglutination" is reversible may possibly be attributable to an increase in the acidity of the clusters; the result of the carbon dioxide produced by the tremendously active spermatozoa. In alkaline medium the carbon dioxide would be neutralized.

[2] In the measurement of the strength of "egg water" the method of F. R. Lillie, (namely considering that dilution of "egg water" that gives a visible "agglutination" as unity), is adopted. Reference is made to the papers of Lillie, F. R., and Fuchs, H. M., for a detailed description of the methods and precautions employed in this type of experimentation.

[3] According to T. B. Robertson, "when spermatozoa are washed in 3/8 M $SrCl_2$ and then in blood serum, they gain an added potency in fertilizing." (Robertson, T. B., 1913, p. 128.) The same treatment agglutinates (*ibid.*, page 71) (and also cytolyzes) (*ibid.*, p. 91) the eggs of the sea urchin.

When sperm are added to egg water, their activity is tremendously increased. If they are then immediately transferred to the ripe eggs of the same species in sea water it is to be supposed that their fertilizing power will for a very short time be at least as great, if not greater than the fertilizing power of less active sperm. The experiments by Fuchs seem to have been conducted in this way. The experiments which are recorded in Table XIV. were carried out in such a way as to make the time during which the sperm were in the "egg water" as short, and the volume of sea water in which the sperm were added to eggs as great, as possible. A few experiments appear to agree with those of Fuchs in that the "egg water" increased the fertilizing power of the sperm. The results are irregular, however, for if the concentration of the "egg water" is too great, or if the time during which spermatozoa are in the "egg water" is too long, so that the activity of the spermatozoa is decreased, the fertilizing power of spermatozoa is not so great as is that of spermatozoa that have been in sea water. This seems to have been the case in the experiment of Lillie quoted by Fuchs; in which "to five watch glasses containing each eight drops of water or of different concentrations of egg-extract were added three drops of 'opalescent' sperm-suspension. After 12 minutes, a drop of a suspension of fresh eggs was added to each.' 5 per cent. of the eggs in the water segmented, but none of those in the four different concentrations of extract" (Fuchs, H. M., 1915, p. 275; Lillie, F. R., 1913, p. 558). It will be noted that in this experiment of Lillie's insemination took place in the egg extracts. Repeating this procedure as nearly as possible, Fuchs was able to obtain higher percentages of fertilization in his "extracts" than in the sea water. This difference in the effect of egg "extracts" upon the fertilizing power of spermatozoa is probably due to the relative concentration of the "extract" and the sperm, and to the absolute strength of the former. For, as Schücking early observed, although spermatozoa are stimulated by low concentrations they are "lamed" (that is, their activity is temporarily decreased) by

TABLE XIV.

THE EFFECT OF "EGG WATER" UPON THE FERTILIZING POWER OF SPERMATOZOA.

Experiment 8.[1]

Concentration of Suspension	Concentration at Insemination.		Age of Sperm.		Approximate Concentration of "Egg Water."							
	Drops of Sperm.	Vol. of Sea Water.	Hrs.	Min.	640.	128.	64.	16.	8.	4.	1.	0.
					Percentage of Eggs Fertilized by Sperm.							
0.04%	I	50 c.c.	0	0				44		59		12
			0	5				17		9		14
	I	200 c.c.	0	0				18		52		23
			0	5				36		11		9

Experiment 12.

Concentration of Suspension	Drops of Sperm.	Vol. of Sea Water.	Hrs.	Min.	640.	128.	64.	16.	8.	4.	1.	0.
0.04%	I	50 c.c.	0	2			98		87		86	78
			0	5			41		76		58	37
			0	10			75		34		10	77
	8	50 c.c.	3	10			22		18		25	14

Experiment 11.[2]

Concentration of Suspension	Drops of Sperm.	Vol. of Sea Water.	Hrs.	Min.	640.	128.	64.	16.	8.	4.	1.	0.
0.04%	I	50 c.c.	0	0		19				11		16
			0	5		15				21		10
			0	10		11				38		

Experiment 16.

Concentration of Suspension	Drops of Sperm.	Vol. of Sea Water.	Hrs.	Min.	640.	128.	64.	16.	8.	4.	1.	0.
0.013%	I	5 c.c.	0	3	48		53	52		42	45 81	27
			I	22	43		62	10		8	6	11

Experiment 5.[3]

Concentration of Suspension	Drops of Sperm.	Vol. of Sea Water.	Hrs.	Min.	640.	128.	64.	16.	8.	4.	1.	0.
0.04%	8	5 c.c.	0	5				49		36	28	19
			0	10				21		26	6	2
			I	00				37		27	8	4
			2	00				42		8	3	2
			5	15				0		0	0	0

Experiment 10.

Concentration of Suspension	Drops of Sperm.	Vol. of Sea Water.	Hrs.	Min.	640.	128.	64.	16.	8.	4.	1.	0.
0.04%	I	50 c.c.	0	3			68	75		95	94	100
			0	8			89	10		83	21	94
			3	50			87	10		61	67	62

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[1] In Experiment 8 the strength of the "egg waters" was as 3 to I, but the "egg water" gave a 60-second reaction.

[2] In Experiment 11 the strength of the "egg waters" was as 125 to 5.

[3] In Experiment 5 the strength of the "egg waters" was as 16:4:I.

higher concentrations of egg "extract."[1] In certain of the experiments that are recorded the "fertilizing power" of the sperm was increased in certain concentration of "egg water" but decreased in greater concentration.

TABLE XV.

THE EFFECT OF "EGG WATER" UPON THE LENGTH OF LIFE OF SPERMATOZOA.

Experiment 17.[2]

Concentration of Suspension.	Concentration at Insemination.		Age of Sperm.		Approximate Concentration of "Egg Water."									
					1280.	640.	128.	64.	32.	16.	8.	?.	2.	0.
	Drops of Sperm.	Vol. of Sea Water.	Hrs.	Min.	Percentage of Eggs Fertilized by Sperm.									
0.013%	2	5 c.c.	1	0		38	57	76						7
Experiment 13.[3]														
0.04%	8	5 c.c.	0	2	100		100			100			100	100
			1	0	100		100			99			96	91
			2	2	98		91			64			42	60
			3	40	99		82			60			26	30
			5	0	95		40			7			8	10
			7	0	54		8			1			2	2
Experiment 19.[4]														
1.0%	1	6.7 c.c.	19	40		66	97			99				0
			24	35		16	77			100				0
			43	40		0	0			0				0

Where the relative concentration of the "egg water" and sperm is such that the activity of the sperm is decreased, the length of life of the sperm, as measured by their ability subsequently to fertilize the ripe eggs of the same species in sea water, is greater than is that of sperm that exhibit constant activity in sea water for an equal length of time. This is to be expected since the activity of spermatozoa is limited (Cohn, E. J., 1917). Whereas the fertilizing power of sperm that have been in "egg water" of various concentrations for but a few moments is often smaller than that of sperm that have been in sea water, the fertilizing power of the latter is soonest lost. Schücking reports an experiment of this kind. "Wenn einer grösseren Spermienmenge ein geringes Quantum Eisubstanz zugesetzt war, so dass die Samenfäden nur gelähmt wurden, so konnten die Spermien noch nach 12 Stunden durch Zusatz von Seewasser wieder beweglich und befruchtungsfähig gemacht werden, während die in Seewasser gebrachten Spermien je nach der Temperatur nach fünf bis acht Stunden abgestorben waren" (Schücking, A., 1903, p. 59).

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[1] All of the properties of "egg water" and "egg extract" are not the same. Sperm are, however, activated and agglutinated by both.

[2] The actual strength of the "egg water" used in Experiment 17 is not known. In all three suspensions, however, it gave at least a three minute agglutination.

[3] An egg extract was used in Experiment 13.

[4] An egg extract was used in Experiment 19. The strengths of the egg extracts were as 1:5:25. The weakest gave a 70-second agglutination.

Since the length of time that "egg water" preserves the life of sperm depends upon the relative concentration of the "egg water" and of the sperm, and since the ability of "egg water" to preserve the life of the sperm depends on the decreased activity of the sperm that follows the initial activation, the sooner the sperm become non-motile (or exhibit decreased activity) the longer the span of their life. It is therefore obvious that a very concentrated "egg water" will often be less effective in preserving their life than a less concentrated "egg water." In Table XV. such conditions evidently obtained. Distilled water extracts of eggs (made isotonic by the addition of sea water that had been concentrated by evaporation) such as were employed by Schücking were used in Experiments 13 and 19. Egg extracts can be obtained with very great "agglutinating strength."

In the experiments reported in Table XIV. the sperm suspensions were, for the most part, made in weaker egg waters. (The egg waters employed in different experiments were probably not of exactly equivalent concentration. The concentrations reported are only approximate.) Moreover sperm was added to eggs in large volumes of sea water. Under such conditions the length of life of spermatozoa, as measured by the loss of fertilizing power, is relatively short.

A large part of the effect of "egg water" in preserving the life of spermatozoa is attributable to the hydrogen ion concentration of these solutions.[1] Lillie, F. R., remarked: "That the sea water which has stood over eggs combines both the effects of an acid, (aggregation) and also an alkali (agglutination) on the spermatozoa" (Lillie, F. R., 1913, p. 549). The acidity is due to the carbon dioxide that the eggs give forth into the sea water together with any other substances that they may secrete. This carbon dioxide may be removed, and the hydrogen ion concentration of the "egg water" decreased. In that case the ability of the "egg water" to preserve the life of the sperm is much decreased, but is still greater than is that of sea water of exactly the same hydrogen ion concentration. An experiment comparing the effects of egg waters and of sea waters of different hydrogen ion concentrations illustrates this relationship.

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[1] Schücking, A., also ascribed this property of egg "extracts" to their acidity. The acidity in the case of his distilled water "extract" of Echinid eggs he believed to be due to mono-sodium and mono-calcium phosphate. The acidity in these experiments was due to the carbon dioxide production of the eggs, for the "egg water" of eggs that had stood in sea water for but short periods of time was used.

TABLE XVI.

THE EFFECT OF "EGG WATER" AT DIFFERENT HYDROGEN ION CONCENTRATIONS UPON THE LENGTH OF LIFE OF SPERMATOZOA.

Experiment 217.[1]

Concentration of Suspension.	Time of Insemination.	Age of Sperm.		Egg Water.		Sea Water.	
				CO_2 Tension.		CO_2 Tension.	
				1.1.	0.8.	1.1.	0.3.
				Hydrogen Potential.		Hydrogen Potential	
				7.5.	7.7.	7.5.	8.0.
		Hrs.	Min.	Percentage of Eggs Fertilized when One Drop Sperm Added to Five Drops Eggs in 5 C.c. Sea Water.			
0.1%	2.45	0	0				
	3.00	0	15	100	100	100	98
	5.00	2	15	100	97	85	17
	6.00	3	15	100	96	70	8
	7.00	4	15	100	65	15	0
	8.00	5	15	100	47	14	0
	9.15	6	30	91	13	1	0

That the ability of "egg water" to preserve the life of spermatozoa is greater than is that of sea water of exactly the same hydrogen ion concentration is probably in large measure due to the higher carbon dioxide and hydrogen ion concentration that is soon reached as a result of the tremendous activity of spermatozoa in "egg water."[1]

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[1] Preparation of egg water: 3 c.c. of eggs that had been strained into sea water at 10 A.M. were allowed to stand in 75 c.c. of sea water until 12.30 P.M. 70 c.c. of the supernatent fluid were then decanted and divided into two 35 c.c. solutions. The hydrogen potential was 7.5, which corresponds to a CO_2 tension of 1.14 mm. The CO_2 tension of the one solution was slightly decreased by passing room air through it until the hydrogen potential was about 7.65 and the CO_2 tension 0.85 mm.

After removal of the carbon dioxide, "egg water" can still agglutinate sperm.[2]

Schücking also found that: "Zwei Tropfen des nicht dialysirten sauren Extracts oder ein bis zwei Tropfen des Rückstandes vom Dialysat, mit einem Tropfen 3 per cent. iger Na_2CO_3 Lösung und fünf Tropfen destillirten Wassers agglutinirten das Sperma noch, obgleich sie neutrale Reaction zeigten" (Schücking, A., 1903, p. 61). Accordingly spermatozoa that are added to egg water, where they are very active, produce a large amount of carbon dioxide. This is also true when spermatozoa are put into alkaline sea water. Unlike the former case, however, the carbon dioxide produced is not so effectively neutralized in the case of egg water, and the acidity of the solution rapidly increases. This is followed, as has been shown, by a decreased activity on the part of spermatozoa and a consequent increase in the length of their life.

The effect of egg water upon the fertilizing power of spermatozoa, is, therefore, not so very dissimilar to the effect of the other substances that have been studied, and can best be understood by analyzing the effect of egg water upon the physiological condition of spermatozoa. That spermatozoa are activated to an exceptional degree by a secretion from the egg has clearly been demonstrated by the work of all of the investigators who have been quoted. It may be that the fertilizing power of spermatozoa that are added to eggs in sea water during this period of activation is increased in much the same way that the fertilizing power of spermatozoa is increased for a short period of time by decreasing the hydrogen ion concentration (increasing the alkalinity) of sea water. If the sperm are added to eggs after they have become inactive, the percentage of eggs that are fertilized depends upon the degree of inactivity and the degree of reactivation (such as is brought about by a transfer to sea water without a high carbon dioxide tension) which the experimental conditions afford. The sooner the sperm become inactive and the more completely inactive they become the longer their life. Thus, excepting for the initial period of activation, the effect of "egg water" upon the length of life of the spermatozoön is essentially that of a solution of optimal hydrogen ion concentration. The hydrogen ion concentration of the "egg waters" that is most effective in preserving the life of the sperm is precisely that of the acidified sea water that best subserves the same function. In the "egg water" this hydrogen ion concentration is reached as a result either of the carbon dioxide produced by eggs and eliminated into the "egg water" or that produced by spermatozoa during the period of their greatest activity.

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[1] The writer is aware of several other possible interpretations of the observation, that (*a*) the ability of "egg water" to preserve the life of the sperm is greater than is sea water of exactly the same hydrogen ion concentration and that (*b*) a very concentrated "egg water" will often be less effective in preserving life than a less concentrated "egg water." For if the "agglutinating substance" be protein in character, as the investigation of Schücking (Schücking, A., 1903) (who found the "agglutinating substance" in the undializable fraction of an egg extract) and of Richards & Woodward (Richards, A., and Woodward, A. E., 1915) suggests the "Studies in the Fertilization of the Eggs of a Sea Urchin (*Strongylocentrotus purpuratus*) by Blood-Sera, Sperm, Sperm-Extract, and other Fertilizing Agents" of T. B. Robertson (1912) may possibly explain these observations. He observed that the "potency of the serum" in initiating either the cytolysis or the development of the egg "obtains a maximum at a dilution of about 1/16" and that "serum of this dilution frequently agglutinates the eggs, causing them to aggregate in large clumps within a few seconds" (Robertson, T. B., 1912, p. 71). In higher concentration the proteins inhibit the imbibition of water by the eggs.

[2] That the "agglutination" phenomenon is not independent of the hydrogen ion concentration of the solution has been shown by Loeb. In acid solution where the sperm are inactive, the "agglutination" cannot occur. The "agglutination" is strongest in solutions slightly more acid than is ordinary sea water but "the clusters" disappear the more rapidly the more alkaline the solution.

XII. Summary.

1. The total available energy of spermatozoa, as measured by total carbon dioxide production, is a constant.

2. The rate at which the total available energy is expended is a function of the activity and an inverse function of the length of life of spermatozoa.

3. The activity and the length of life of spermatozoa is a function of the temperature, and of the hydrogen ion concentration of the suspension. Up to a certain point decreasing the temperature or increasing the hydrogen ion concentration decreases the

activity and increases the length of life of spermatozoa. Lower temperatures or greater acidities destroy spermatozoa, and the more so the longer they are subjected to these abnormal media. Conversely, within limits, decreasing the hydrogen ion concentration or increasing the temperature increases the activity and decreases the length of life of spermatozoa. Further increase in temperature or in alkalinity irreversibly agglutinates spermatozoa.

4. The ability of spermatozoa to fertilize eggs of the same species is a function of their activity (as measured by their carbon dioxide production, or its converse, their length of life). Spermatozoa lose their power to fertilize as a function of the time of insemination, and of the dilution and of the hydrogen ion concentration of the suspension. If spermatozoa that have been in the suspension for but a short time are added to eggs in a large volume of sea water a decrease in the hydrogen ion concentration of the sperm suspension (that is, an increase in the alkalinity) increases the activity and the fertilizing power of the spermatozoa. Such spermatozoa will, however, lose their power to fertilize when transferred to ripe eggs in sea water long before spermatozoa that have been relatively inactive in more acid sperm suspensions.

5. If spermatozoa that have been at the same hydrogen ion concentration are added to eggs in sea water at the same time, increasing the concentration of spermatozoa increases the percentage of eggs that are fertilized. This may be explained either by assuming that in the greater concentration more spermatozoa will on the average arrive at the periphery of the egg with sufficient action (having the dimensions of energy × time) to initiate development, or by assuming that mass action of spermatozoa may be effective in initiating the development of the sea urchin egg. The latter explanation has been suggested by Glaser, who pointed out that the further assumption that more than one spermatozoön was necessary "to carry out the bi-parental effect" was not involved.

6. Increasing the carbon dioxide concentration increases the hydrogen ion concentration of the suspension, decreases the activity of spermatozoa and increases the length of their life.

Sea water of the optimum carbon dioxide concentration for increasing the length of life of spermatozoa is also of the optimum hydrogen ion concentration. It is possible, however, that carbon dioxide affects the physiological condition of spermatozoa otherwise than by means of ionized hydrogen.

7. That decreasing the oxygen concentration of sea water or decreasing the oxidations of spermatozoa by adding KCN to sea water increases the length of their life has been reported by Drzewina and Bohn. Experiments confirming their results and demonstrating that under such conditions sperm are relatively inactive are reported.

8. Spermatozoa increase the carbon dioxide and, therefore, the hydrogen ion concentration of concentrated sperm suspensions to the optimum for decreasing their own activity in a very short time. In such suspensions sperm live for a very long time. McClendon has recently estimated that respiration in sea water that raises the carbon dioxide and the hydrogen ion to a concentration approximately equal to that which is most effective in prolonging the life of a sperm suspension, uses up all of the available oxygen. It is therefore suggested that high carbon dioxide and hydrogen ion concentration and low oxygen concentration are the environmental conditions that obtain in a concentrated sperm suspension. These conditions approximate those of ripe sperm in the testes very much more closely than they do those of sperm that are shed in the ocean.

9. The addition of beef broth to sea water increases the length of life of the spermatozoa. Gemmill suggested that beef broth furnished "artificial nutrition." It is pointed out that the addition of beef broth to sea water increases the hydrogen ion concentration and decreases the activity of spermatozoa.

10. Sea water that has contained the eggs of the sea urchin, *Arbacia*, tremendously activates spermatozoa. It may be that the fertilizing power of spermatozoa that are added to eggs in sea water during this period of activation is increased in much the same way that the fertilizing power of spermatozoa is increased for a short period of time by decreasing the hydrogen ion concentration (increasing the alkalinity) of sea water.

Subsequent to the period of activation spermatozoa become inactive in egg water. Such egg water has a high hydrogen ion concentration. This is reached as a result either of the carbon dioxide produced by eggs and eliminated into the sea water, or by spermatozoa during the period of their greatest activity. If spermatozoa are added after they have become inactive, the percentage of eggs that are fertilized depends upon the degree of inactivity and the degree of reactivation (such as is brought about by transferring spermatozoa to sea water without high hydrogen ion or carbon dioxide concentration) which the experimental conditions afford. The sooner spermatozoa become inactive and the more completely inactive they become, the longer their life. The hydrogen ion concentration of egg water that is most effective in preserving the life of spermatozoa is precisely that of acidified sea water that best subserves the same function.

Other effects of egg water upon the physiological condition of spermatozoa have not been considered in this investigation.

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BIOLOGICAL BULLETIN

CRITICAL NOTES ON THE PRESENT STATUS OF THE LENS-PROBLEM.

E. I. WERBER.

1. Introduction.

The present communication is an attempt to arrive at a common understanding of the nature of the difficulties that stand in the way of the solution of a problem to which for over a quarter of a century have been devoted the best efforts of a number of biological observers.

One phase of this problem, although at one time rather near solution, has since, owing to—apparently unavoidable—technical difficulties of the experimental method, become ever more complex. In the following it is my intention to show that this complexity is an artificial one and that it practically disappears on careful sifting of evidence. Owing to this seeming complexity, however, an originally correct interpretation has lately been giving way to one that not only is unwarranted, but has already occasioned even some very improbable and fruitless phylogenetic speculations (cf. for instance Becher, '12).

Anticipating to return to the subject of this communication in a more comprehensive publication in the near future I hope I may be pardoned for incompleteness in considering the pertinent literature at the present time. Only such contributions can here be considered, as from my point of view seem to have the most significant bearing on the problem.

2. The Origin of the Primary Lens—in Ontogeny.

As well known Spemann ('01, '03) was the first to furnish experimental evidence for the correctness of the opinion—advanced simultaneously also by Herbst ('01)—that the lens of the vertebrate eye is dependent in its origin and differentiation upon the contact of the optic vesicle with the supra-ocular epidermis from which it arises. It will be recalled also that this view was contested by Mencl ('03, '08), King ('05) and Stockard ('10) and that Spemann himself has in later papers ('08, '12) modified his opinion and abandoned his generalizations. He now thinks that more extended experiments in which various methods were employed on several frog species prove that in some species the lens cannot develop if the optic vesicle fails to come into contact with the overlying epidermis while in one species the epidermis is capable of giving rise to lentiform bodies and even well-differentiated, unmistakable lenses without such contact.

Such conditions would, indeed, be so contrary to expectation that it well behooves us to inquire into the validity of Spemann's self-contesting evidence as well, as of the evidence brought forth by Mencl, King and Stockard for the independent development of the lens.

I have already (Werber '16*c*) pointed out some possible sources of error which would invalidate the conclusions of these authors and on re-reading the entire series of Spemann's interesting papers on the subject I have been struck by the complete absence of any real counter-evidence against the conclusions of his earlier ('01, '03) excellent work.

Spemann's first experiments were performed on *Rana fusca* and consisted in destroying, by pricking with a heated needle or the galvanocauter, the right (presumable) optic pit (foveola optica).[1] As a result he found that no lens was formed, if the entire optic pit had been destroyed or, if deeply buried remnants of the latter which developed into diminutive optic cups failed to reach the epidermis. If, however, such a rudimentary optic cup did come into contact with the epidermis, a lens developed secondarily from the latter.

I am inclined to think that these results practically solved the problem, and for this reason it may perhaps be regarded as unfortunate that Spemann's well-warranted generalizations have been the subject of ill-founded criticism by Mencl ('03). For while the latter was very aptly met by Spemann ('03), it apparently occasioned him to enter upon a great many experiments on the subject which, though beautifully conceived and skillfully executed, have, owing to the pitfalls of a treacherous material, yielded fallacious results.

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[1] For a description of this earliest discernible eye primordium cf. Eycleshymer ('93 and '95) and Froriep ('06).

The first experiments which have occasioned Spemann ('07) to modify his views were performed on *Rana esculenta.* They consisted in the excision of the "right anterior half of the brain primordium" from the wide open medullary plate.[1] As a result he observed that a lens has developed in spite of the absence of an eye on the side operated upon. Like results were obtained also when the optic pit was destroyed at the same stage of development with a heated needle. The results were apparently entirely independent of the method of operation. In both cases, however, they differed from those obtained in *Rana fusca,* in which, as we have seen, no lens was formed when the optic anlage was at this stage destroyed by pricking with a heated needle, while the excision experiment in this species, according to Spemann ('07) "ergab ein sehr unsicheres Resultat." In one out of four examined embryos Spemann ('12) observed "ein kleines Bläschen, welches eine Linsenanlage sein könnte."

Why such divergent results in experiments on two species of the same frog genus with the same methods and at the same stage of development? Spemann concludes from his experiments on *Rana esculenta* that in this species the "lens-forming cells" of the embryonic epidermis are capable of independent development in the absence of an optic vesicle, while *Rana fusca* lacks this ability for self-differentiation.

The same experiments on *Bombinator pachypus* (at the same stage of development) gave less definite results. While no lens developed in this species on the side operated upon, Spemann observed in some few instances structures which he could not with certainty identify. Experiments on embryos of the same species at a later stage of development, *i. e.*, removal of the optic vesicle, the overlying epidermis being previously raised up and reflected from it, gave also ill-pronounced results. In a number of instances Spemann observed in such embryos on the side operated upon a thickening of the epidermis in the region where a lens normally should have developed, which he is inclined to consider as an abortive attempt at lens-formation. From these experiments Spemann tentatively concludes that *Bombinator* in regard to the power of independent lens-formation occupies a sort of intermediate position between *Rana fusca* which lacks this ability and *Rana esculenta* which, according to him, possesses it in a decidedly marked degree.

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[1] In my 1916c paper several, otherwise wholly insignificant, errors have unfortunately crept in into the references made to these excision experiments in *Rana esculenta* and to King's pricking experiments. In both cases it should read "optic pit" or "optic anlage" instead of "optic vesicle."

Are these conclusions justified? I am strongly inclined to think that they are not, the results on which they are based being very inconclusive. Moreover, on careful examination of the excision experiments in *Rana esculenta* the impression is gained that some fragments of optic substance were left after the excision of the anterior half of the primordium of the brain hemisphere. Thus, for instance, in his 1912 paper in Fig. 6 the smaller one of the two observed lentoids is in close apposition to a "ganglion." May not this ganglion rather be a small group of retinal cells? In Figs. 8 and 9 small groups of tapetum nigrum cells may be observed, and Spemann (p. 22) admits "dass vom Augenbecher vielleicht kleine Reste vorhanden seien." In Fig. 8 the tapetum-cells are very close to the lentoid, while Fig. 7 also suggests that dispersed tapetum nigrum was responsible for the origin of its "Linsenbläschen." Instead of concluding, as does Spemann, that the lentoids of Figs. 6 and 9 are due to dispersion of "lens-forming cells" it might perhaps be safer to think that in these experiments fragments of potential optic-cup substance were dispersed and stimulated the differentiation of lentoids by chancing to come into contact with the epidermis.

Again, in Fig. 10 representing a cross-section through an embryo in which the anterior part of the anlage of the right brain hemisphere has been destroyed by a heated needle, the lentoid observed is in close proximity to a rudimentary optic cup (oc′, Fig. 10), which clearly demonstrates the fact that the attempted elimination of the anterior part of the brain primordium may often be illusory, fragments of optic-pit substance remaining after the operation. According to Spemann ('12, pp. 24 and 25) it is easier to eliminate the entire optic anlage in *Rana fusca* than in *R. esculenta*. He attributes this to a difference in the "Konsistenz" between the embryos of the two species. "So ist wohl das Resultat zu erklären, dass der Defekt bei den allerdings nicht sehr zahlreichen Versuchen dieser Art meist entweder zu gross wurde, im ersteren Fall also vom rechten Auge ein Rest erhalten blieb, im letzteren die Linsenanlage mit zerstört wurde."

In my estimation the divergent results obtained both by pricking with a heated needle and by excision in both species would rather seem to point to differences in the location of the optic pits in the neurulae of the two species at the same stage of development. Thus, in *Rana esculenta* they are probably more diffuse and extend more laterally than they are in *R. fusca*. Accordingly, if at this stage the excision of the anterior half of the brain primordium be attempted, it may happen that a small fragment of the optic pit may remain, as in this species it may extend even laterally from the medullary fold. Granting, however, that fragments of potential optic-cup substance do sometimes remain after the operation in this species—and Spemann admits that both in text and in the figures—it is no longer difficult to understand that in such embryos lentoid structures and even well-differentiated lenses may be formed on the eyeless side owing to the "lentogenic stimulus"[1] from the remnant of optic substance on the epidermis.

Spemann does not seem to have considered the results obtained from these operations as very conclusive, if as much can remain of the brain anlage after its attempted elimination as "ein Fragment des Tapetum nigrum," "der vorderste Teil der Vorderhirnanlage, . . . ein dorsales Stückchen Zwischenhirn mit Epiphyse und Plexus chorioideus, jedenfalls entstanden aus lateralen Partien der Medullarplatte, endlich ein Stückchen der linken Hälfte des Mittelhirns" ('12 p. 28). For, not content with these results he sought confirmation in experiments in which several other methods were employed.

Thus, adopting Lewis's ('04) method, he removed (by excision) in *R. esculenta* the optic vesicle after having previously raised up and reflected the overlying epidermis. The latter was then affixed in its place where it healed and in two embryos gave rise to "ein deutliches Linsenbläschen" in the absence of the optic cup. Spemann considers these lens-buds as evidence of the ability for independent lens formation in *R. esculenta*.

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[1] Cf. Werber '16c.

The validity of this conclusion, however, seems questionable to me in view of a well-nigh uncontrollable source of error which Spemann himself has pointed out ('12, p. 42). For he states expressly that the lower stratum of the epidermis (the "Sinnesschicht") adheres so firmly to the eye vesicle that on attempting to separate the epidermis from the latter usually the upper layer (the "Deckschicht") is raised up, while the lower one remains attached to the optic vesicle. Regardless of Spemann's skill in such delicate operations and the precautions he has taken to separate the entire epidermis from the optic vesicle it was apparently impossible always to avoid minute fragments of the latter remaining attached to it. And the two cases in which he obtained "independent" lens-buds have possibly resulted from just such an unsuccessful operation, while in the embryos in which no such structures were recorded on the side operated upon the operation was apparently faultless.

Spemann assumes that in his unsuccessful experiments of this series, *i. e.*, where no "independent lenses" were recorded, the "lens-forming cells" of the lower stratum of the epidermis were removed with the optic vesicle to which they remained attached. I can see no valid reason for the assumption of cells predetermined to form lenses. The often-raised argument of the cyclopean eye in which the lens is formed from epidermis that normally does not develop into this structure, and Lewis's ('04, '07a and b) experiments in which the formation of lenses from strange ectoderm and even from ectoderm of another species was demonstrated to be possible—an experiment successfully repeated by Spemann himself by transplanting the bared optic vesicle of *Rana esculenta* under the ventro-abdominal epidermis of *Bombinator*—speak very decidedly against this assumption.[1]

Moreover, why should just the species *Rana esculenta* form such a strange exception as to possess such so early specialized regions of the epidermis? I am under the impression that the apparent fallacy of Spemann's conclusions from this series of experiments is most likely due to the circumstance that while he detected an important source of error, he unfortunately, mistook the nature of the error. For had he considered the possibility that fragments of the optic vesicle too small to be detected with the binocular dissecting microscope and even so minute as not to be able to differentiate into histologically discernible structures, may suffice to induce the lentogenic reaction in the epidermis to which they remained attached, he would probably have considered the "unsuccessful" experiments of this series as successful and vice versa. Accordingly, instead of believing that in the two "successful" cases the "lens-forming cells" had not (or not entirely) been removed, he might have concluded that owing to the above-mentioned difficulty some remnants of optic substance have stimulated the development of the lens-buds demonstrated in Figs. 37, 38 and 38a.

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[1] Mencl ('08, Fig. 3) described an "intracerebral eye" (intracerebral cyclopia) whose lens is derived from the epithelium of the mouth. The ability of this part of the ectoderm to give the "lentogenic reaction" is demonstrated also by the lentoids of the mouth which I have recorded (Werber '16c).

The results of other experiments performed by Spemann ('12) which may now be examined would also seem to lend support to his former views rather than to his present ones. One of these experiments consisted in transplanting abdominal epidermis over the bared optic vesicle in *Rana esculenta*. The flap of epidermis used for the purpose was previous to its transplantation "von etwa anhaftenden Mesodermzellen sorgfältig gereinigt." The result was negative—no lenses or lens-like structures were formed by the strange epidermis owing, as Spemann thinks, to the circumstance that the "primären Linsenbildungszellen" were contained in the flap of supra-ocular epidermis which had been removed. However, four cases were recorded in which the epidermis transplanted over the eye presented an appearance different from that of the epidermis of the immediate surroundings. And in Fig. 63 there is "eine deutliche Linsenanlage (L) vorhanden, ein kleines, dickwandiges Epithelbläschen, welches mit der transplantierten Rumpfhaut über dem Auge innig verbunden ist." ('12, p. 55). Spemann mentions also another embryo of this series in which he observed an indication of lens-formation on the side operated upon (Fig. 58), but assumes that in both cases "primäre Linsenbildungszellen" remained attached to the optic vesicle owing to faulty technique in the removal of the supra-ocular epidermis.

Might we not with at least just as much justification assume that in the embryos in which no lenses were formed from the transplanted epidermis this was due to lack of immediate contact between the optic vesicle and the epidermis, because mesodermal cells were attached to the latter? For, regardless of the most painstaking care I think it is impossible to be sure that the epidermis has been cleaned of these cells entirely with the aid of such low magnifications as even the highest power lenses of the binocular dissecting microscope. It is, besides, difficult to understand why the optic vesicle of this species should be able to induce lens-formation from ventro-abdominal epidermis of *Bombinator* and not from the abdominal epidermis of an embryo of its own species.

Whatever one might think of the results of these experiments, they certainly cannot be regarded as conclusive in favor of Spemann's present views.

Two more series of experiments on *Rana esculenta* may now be considered the results of which Spemann interprets in favor of the independent development of the lens in this species. The method employed in these experiments was autoplastic transplantation and the part transplanted was the "primären Linsenbildungszellen."

In the first (a) series a more or less rectangular piece of supra-ocular epidermis was detached during or immediately after the closure of the medullary tube and turned about 180°, care being taken that no fragments of the optic vesicle be left on it. In the second (b) series the operation differed only in one point, viz., that in detaching the supra-ocular epidermis a fragment of the optic vesicle remained attached to it and owing to the inversion of the piece of epidermis (by turning it about 180°), it became transplanted into a posterior region (otic capsule).

In the experiments of the first (a) series great technical difficulty was encountered and only in four cases was the separation of the epidermis from the optic vesicle "anscheined einwandsfrei." In three of these cases the eye of the operated side had "eine deutliche Linse," while in the fourth case where, owing to the operation, the otic labyrinth came to lie between the epidermis and the eye, the latter lacked a lens. No lens, however, developed in any of these embryos caudally from the eye, as might have been expected, had that part of the epidermis ("primäre Linsenbildungszellen") been capable of giving rise to a lens by self-differentiation.

These cases (although so few in number) point decidedly, I think, to the inability of supra-ocular epidermis in *R. esculenta* to differentiate a lens in the absence of a stimulus from the eye vesicle. Spemann, however, prefers the tentative explanation "dass nach Auslösung einer Linsenbildung durch's Auge die spontane Entwicklung einer zweiten Linse aus den primären Linsenbildungzsellen unterbleibt." He is inclined to consider even the possibility "dass bei diesen Experimenten die Linsenbildungszellen nicht weit genug nach hinten gebracht worden waren und dass entweder sie selbst oder ihre nächste Umgebung die Linse des stehengebliebenen Auges geliefert haben." As support for the latter possibility he regards the observation, "dass die Linse in allen 3 Fällen im hintersten Winkel des etwas deformierten Augenbechers liegt und einmal sogar etwas in die Länge gezogen ist" ('12, p. 64). Is it not more probable that this distorted relation between the lens and the optic cup is due to the very fact that the latter was deformed owing to the operation? From my observations on many teratophthalmic *Fundulus* embryos I know that this is very often the case in deformed eyes.

One cannot escape the impression that Spemann by considering such possibilities and by regarding "diese Versuche . . . als misslungen und die Frage als unentschieden" has—apparently unconsciously—missed the only obvious conclusion, viz., that *Rana esculenta* possesses no early predetermined lens-forming cells and that a stimulus from an optic cup or from some of its parts is necessary to induce the differentiation of a lens from the epidermis in embryos of this species as much as in *Rana fusca*.

Let us now consider the second (b) series of these experiments in which, as will be recalled, a fragment (the tip, "Kuppe") of the optic vesicle was left attached to the inverted epidermis.

Observations were made on seven embryos operated upon in this manner. In all of them the anterior eye fragment lacks the lens, while the posterior one in five embryos stimulated the formation of a lens which "*kann* so vollkommen entwickelt sein wie die normale" (p. 67). In one of these embryos the posterior eye fragment possesses even two lenses which Spemann is inclined to regard as being due to mechanical separation of the "Linsenmaterial" into two parts, although it would seem more probable that the duplication of the lens in this case may (owing to the operation) be due to mechanical histolysis of the eye fragment which thus came into contact with the overlying epidermis in two places.

From these observations Spemann (p. 67) concludes that "die weitgehende Determination der primären Linsenbildungszellen von *Rana esculenta*, welche sie zu selbstständiger Entwicklung befähigt, bringt es wohl mit sich, dass die übrigen Epithelzellen, auch die der nächsten Umgebung, nicht mehr imstande sind, auf einen Reiz des Augenbechers mit Linsenbildung zu antworten."

These conclusions cannot be considered as warrantable. Moreover, the results permit of an interpretation which, I believe, is founded upon a much greater probability. It must be remembered that at the stage of development at which the operations were performed, it is only the tip ("die Kuppe") of the eye vesicle that is protruding from the head. If that protruding part be removed, the epidermis transplanted over the resulting gap may, owing to the latter, fail to come into contact with the deeper part of the eye even on subsequent growth of the latter owing to the obstruction formed by mesenchyme cells which may grow into the gap before such contact can be effected. Spemann's Fig. 67*b* very forcibly suggests just this possibility. On examining the anterior eye fragment of this figure one can observe that it is not in contact with the epidermis. More significant, however, is the observation in its lumen of a blood vessel and of some mesenchyme cells which also fill the space between it and the epidermis.

Our interpretation of these apparently vexing cases receives further support from the very fact that in the (a) series of these experiments where the "Kuppe" of the optic vesicle had not been removed, the latter was able to stimulate the formation of lenses, because there being no gap between it and the epidermis transplanted over it, there was no chance for an obstruction of its contact with the latter by ingrowing mesenchyme. It is, besides, needless to say that it is difficult to understand just why Spemann in the experiments in which the epidermis was transplanted over a defective eye vesicle assumes a sharp predetermination to form lenses and not so, if transplanted over an uninjured eye vesicle.

Summarizing briefly *we find that critical sifting of the results of Spemann's experiments on Rana esculenta discloses no warrantable evidence whatsoever for the presence in this frog species of an early predetermined lens-forming part of the epidermis capable of differentiation into a lens without the stimulus from an optic vesicle or at least a fragment of it.*

Practically all of these experiments were performed by Spemann also on *Bombinator pachypus.* The results obtained in this species differed according to Spemann ('07, '12 ff) from those obtained in the pricking experiments in *Rana fusca* and also from the above-noted results in *Rana esculenta.* They indicate, Spemann believes, that *Bombinator* in regard to the ability to form a lens by self-differentiation occupies a position intermediate between *Rana fusca* and *Rana esculenta.*

It is evident, I believe, that were this really so, *i. e.*, if the supra-ocular epidermis of *Bombinator* possessed a certain degree of this ability and only needed the stimulus from the optic vesicle as a complementary aid for the differentiation of a lens, a serious stumbling block would here confront every attempt at the solution of the lens-problem.

Fortunately, however, Spemann's observations seem to call for an entirely different interpretation of the results.

Thus in the excision experiment (right foveola optica) the latter prove very decidedly, I believe, that no lens can be formed in the absence of an optic anlage or on its failure to come tinto contact with the overlying epidermis. For, out of the forty-six embryos operated upon in twenty of them in which an optic fragment remained (owing to incomplete excision), a lens developed on the side of operation. Of the remaining twenty-six cases one is uncertain, for here a structure was observed which might perhaps be a deformed lens-bud, in twenty cases neither an optic cup nor a lens can be observed, while in five cases the optic cup is so small that there was no contact with the epidermis. According to Spemann, however, in two of the latter cases the condition is not clear, one of the embryos possessing (on the side of excision) near the brain "ein dickwandiges Bläschen mit kleinem Lumen," which Spemann is unable to identify, while in the other case the region of epidermis which should have furnished the lens, is "kaum merklich verdickt."

The only warranted conclusion from these results is: *The contact of at least a fragment of the optic anlage is necessary to induce the formation of a lens from the overlying epidermis.* It is unwarranted however, to conclude, as does Spemann (12, p. 38), ". . . dass Bombinator pachypus zwar auch primäre Linsenbildungszellen besitzt, welche von den Epidermiszellen der Umgebung verschieden und zur Umbildung in die Linse vorbereitet sind, dass diese Zellen aber der Mitwirkung des Augenbechers bedürfen, um in Aktion zu treten, zum mindesten in viel höherem Masse als die Linsenbildungszellen von R. esculenta."

The results of other experiments in this species also point decidedly to the incorrectness of Spemann's conclusions. Thus the removal of the optic vesicle during or immediately after the closure of the medullary folds resulted only in a thickening ("Wucherung") of the epithelium in the region where normally the lens should have arisen. In two cases, however, small remnants of the optic vesicle developed into diminutive optic cups lacking a lens, because mesenchyme had grown in between them and the epidermis. Lentoid structures (two) were observed only in one case (Figs. 41*a* and *b*, L′), which are probably due to a stimulus from very minute remnants of the optic vesicle.

These experiments would again seem to *prove only that*, as Lewis ('04, '07*a* and *b*) and Le Cron ('07) have found, *a stimulus from the optic vesicle on the epidermis of sufficient duration is necessary for the latter to give rise to a lens.* They do not prove, however, the presence in this species of early predetermined lens-forming cells requiring the stimulus from an optic vesicle only as a supplement for full differentiation into a lens.

On transplanting ventro-abdominal epidermis over the bared optic vesicle in this species Spemann recorded as a result that in none of the sixteen examined embryos a lens developed from the strange epidermis. From these results he concludes that either the optic vesicle cannot stimulate abdominal epidermis to the formation of a lens or that this part of the epidermis is incapable of responding to such a stimulus.

Here again, however, the same objection is unavoidable which we have raised against the conclusion from the results of the same experiments in *Rana esculenta*, namely that regardless of the experimenter's care mesenchyme cells attached to the transplanted epidermis obstructed a contact of the latter with the optic vesicle.[1]

Very clear ("eindeutig") results were obtained in the experiments in which, as in *Rana esculenta*, a flap of the skin of the head including the supra-ocular epidermis was turned about 180°. In the first (a) series in four out of eight "einwandsfreie" cases the eye cup was not in contact with the epidermis and, naturally, lacked a lens. In the other four cases there was such contact—and the eye possessed a lens.

Accordingly: *There are no lens-forming cells, but epidermis that normally does not give rise to a lens will differentiate into a lens, if brought into contact with the optic vesicle.*

The second (b) series of these experiments (a fragment, the "Kuppe," of the optic vesicle transplanted with the turned epidermis) yielded results very similar to those of the corresponding experiments in *Rana esculenta*.

With but one exception the posterior eye fragment always obtained a lens, which, as Spemann rightly adds, could not have developed in the absence of this fragment of optic-cup substance.

The anterior eye fragment, however, obtained no lens in fifteen out of the twenty examined embryos, while a lens or only a thickening of the epidermis opposite the eye fragment was observed in five cases. In thirteen of the embryos which had no anterior lens Spemann accounts for its lack by the ingrowth of mesenchyme into the space between the optic-vesicle fragment and the epidermis, widened by the expansive growth of the otic capsule which, owing to the transplantation came to occupy this anterior position. He is unable, however, to suggest an explanation for the lack of this lens in two cases, "wo kein Grund für ihr Ausbleiben zu erkennen ist" (p. 73).

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[1] It is significant that in one out of the four cases in which he reports to have observed a "Wucherung" opposite the eye "eine zusammenhängende Schicht sehr dotterreicher Zellen" (p. 60) was conspicuous in that interspace. That shows that it is apparently futile to attempt to clean the abdominal epidermis of all mesenchyme cells. While it is difficult to know just how to interpret these "Wucherungen," they might possibly be due to mechanical distortion during the operation.

From these experiments Spemann ('12, p. 77) concludes that the optic vesicle of *Bombinator* is capable of exerting a specific stimulus not only on the lens-forming cells, but also on other parts of the head epidermis.

This conclusion is, I think, only partly correct, the assumption of "primäre Linsenbildungszellen" being both unwarranted and unnecessary. For leaving out of consideration the two cases (which I should not wish to pre-judge), where no anterior lens was formed while it should have been expected, we can conclude only that its presence in the several other cases was due to the specific stimulus from the anterior eye fragment.

Briefly, the results of this series of experiments also contradict Spemann's opinion of the intermediate position of *Bombinator* between *Rana fusca* and *R. esculenta* with regard to the ability of independent differentiation of the lens.

In *all of Spemann's work* I find only *a confirmation of his initial results and strong support for the generalizations which he made in 1901 and 1903, but no counter-evidence whatsoever that would justify his present ('12) opinion.*

Other evidence for the independent development of the lens brought forth by Mencl ('03, '08), King ('05) and Stockard ('10) is no less illusory.

Mencl ('03) described the head of an anophthalmic component of an anadidymus in *Salmo salar* which possessed two laterally located lenses although there could be observed "keine Spuren von Augenblasen, ja nicht einmal von Anlagen derselben." Both of these lenses were in close apposition to the deformed brain, the larger one even having, owing to its growth, formed a pit in the latter by pressure. Both lenses, however, were so situated that their derivation from lateral parts of the head epidermis was beyond doubt. What is responsible for the origin of these lenses? Mencl suggested (p. 337) that "der, diese zwecklose, wie durch Erinnerung der Epidermiszellen auftauchende Linsenbildung auslösende Faktor ist die Vererbung."

A much more reasonable and, I think, the only correct, interpretation of this case was offered by Spemann ('03) who concluded (p. 464) that the optic vesicles "oder genauer ihr für die Linsenbildung allein in Betracht kommender retinaler Teil nur scheinbar fehlen, indem die Partie der Hirhwand, welcher die Linsen angelagert sind, nichts anderes ist, als die nicht abgegliederte . . . Retina."

Although this interpretation was later abandoned by Spemann "unter dem Druck neuer Tatsachen" ('12, p. 3), my experience with teratological material—and Mencl's case is a teratological one—forces me to lend it unreserved support.

For the sake of clearness in presenting my point of view (based largely on teratological data) the following brief recapitulation of the present stage of our knowledge on the morphogenesis of monsters may be permitted.

It was repeatedly noted by pathologists and teratologists and notably also by Mall ('09) that examination of malformed embryos often discloses evidence of destruction, dissociation and shifting of tissues or parts of the embryo. On examination of a great many experimentally produced monsters in *Fundulus heteroclitus* I ('16*a* and *b*) was able not only to confirm these observations, but also to demonstrate some remarkably striking cases of the effects of such dissociation of parts of the early embryonic primordium and to account for the causes of this process which I have termed blastolysis. This "blastolytic action of the chemically modified environment is . . . a morphogenetic principle common to all terata. . . . Blastolysis either destroys part or all of the germ's substance, or it may split off and disperse parts of the latter" (Werber '16*b*, p. 569).

It is this destruction of tissues, the subsequent elimination of parts destroyed and the resulting dissociation and shifting of parts surviving that in experiments in which eggs are subjected to a chemical modification of the environment, brings about the weirdest malformations of the developing embryos.

All of these deformities are thus clearly *due to a defect* (*or defects*) *of a blastolytic nature.* Not only is this the case in experiments in which the modification of the environment employed is a chemical one, but also, if a physical, *e. g.*, thermic, modification be employed. For, in experiments (not yet published), in which the eggs of *Fundulus heteroclitus* were subjected to the action of a temperature much below the normal, some terata and particularly ophthalmic monsters resulted. The latter are due to loss at an early embryonic stage of parts of ophthalmoblastic material and to like damage sustained by the anterior part of the potential head. Thus anophthalmia results from such loss by the earliest brain primordium of the whole or nearly whole ophthalmoblastic material.

In some anophthalmic embryos such blastolytic optic-cup fragments or even small, fairly well differentiated optic-cups may on microscopic examination of sections be observed either enclosed in the brain between both hemispheres (intracerebral cyclopia, as it were, cf. Mencl '08, Figs. 2 and 3) or in a lateral position as a part of the brain. Such "concealed" remnants of the optic cup may often lack the tapetum nigrum and the histological character of retina, owing to early destruction of some groups of cells which potentially corresponded to the lacking layers of the retina, and may thus give the appearance of a part of the brain. If it happens to come into contact with the epidermis, such "a part of the brain" will stimulate the differentiation of a lens from it.

This, undoubtedly, is exactly the condition in the case described by Mencl in 1903. I have many times observed unmistakable optic cups which lacked some of the histological characteristics of retina and whose structure was very much like that part of the "brain" in Mencl's case (cf. Mencl '03, Figs. 2, 4 and 5, and also '08, Fig. 1), into which the larger one of the two lenses has, as it were, burrowed in. That part of the "brain" is undoubtedly a "verkappte" retina, as Spemann had once suggested.

This, I think, disposes of the first, but most troublesome, case described by Mencl. It might perhaps be added that the asymmetric position of the two lenses[1] in the head of this anophthalmic anadidymus component could not be accounted for by "hereditary reminiscences," while chance contact of remnants of dissociated ophthalmoblastic material of both sides with the epidermis does account for the origin of both lenses as well as for their asymmetrical position. Neither of them has arisen from predetermined, lens-forming cells—"durch Erinnerung," but from indifferent ectoderm in response to stimuli from remnants of potential optic cups on the epidermis.

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[1] "Die linke liegt mehr dorsal und zugleich caudalwärts von der rechten" (Mencl, '03, p. 331).

The other "independent" lenses and lentoids described by Mencl ('08) can even much easier be demonstrated to be due to the same origin—blastolysis and subsequent contact of fragments of optic cup substance with the ectoderm. From his description of sections of the embryos underlying the Figs. 4, 5, 6, 7 and 8 the reader will easily gather that in these cases fragments of optic-cup substance[1] have been profusely dispersed and in places of contact with ectoderm gave rise to lenses or lentoids. Mencl did not appreciate the full significance of his own observations, for referring to such an optic fragment ("Pigmentanhäufung," Fig. 5*X*, he says: "Was dies Gebilde bedeutet, *kann ich nicht entscheiden—das Vorhandensein des Pigments jedoch, sowie die retinaartige Anordnung der Zellen in der benachbarten Hirnwand*[2] lässt die Meinung entstehen, dass es sich dabei um irgend ein Rudiment einer atypisch und selbstständig zur Entwicklung gelangten Augenkomponente handelt" (p. 447). It is, indeed, difficult to understand just why, in spite of these observations, Mencl claims that in these embryos "die selbstständige Entwicklung der Linsen über jeden Zweifel erhaben ist" (p. 447).

I have recently examined sections of a number of deformed Salmonid embryos (of another species) with such "independent" lenses. The publication of the observations made on this material being reserved for a future publication, it may suffice to state that the conditions noted in several of these embryos bore a very striking resemblance to those in the embryos lately described by Mencl ('08). Here, too, subjective interpretation might easily lead to the conclusion that the "eyeless" lenses arose by self-differentiation of the ectoderm. But careful observation and rigid analysis of the noted relations of parts to each other leaves no doubt that all these lenses are products of stimuli from dispersed optic-cup substance.

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[1] "Pigmentanhäufung," "Pigmentbläschen," "Pigmentfleck," "eine homogene, schwarze, längliche Pigmentmasse, welche in die Falte der retina-artigen Hirnwand hineinragt" (Mencl '08, p. 447).

[2] My own italics.

In a *Fundulus* monster described by me in a former paper (Werber '16*c*) there were no eye defects, but owing to a special method optic-cup substance was very profusely dissociated and dispersed through a large part of the head. Owing to this condition a great deal of the head ectoderm and even the epithelium of the mouth, infected, as it were, with such fragments of the optic anlage, responded by the formation of a great number of lentoids. Many more monsters resulting from the employment of the same method were examined in sections and they show very similar conditions. In other experiments in which the method employed was more destructive various eye defects such as monophthalmia or synophthalmia or anophthalmia resulted. On examination of sections through these embryos not only lentoids, but well-differentiated, "eyeless" lenses were frequently observed and in nearly every one of these cases some more or less obvious traces of optic-cup substance can be observed in their immediate neighborhood.

In the same paper I have pointed out that the "independent" lenses which Stockard ('09, '10) described in teratophthalmic *Fundulus* embryos have also undoubtedly resulted from contact of ectoderm with such blastolytic fragments of the eye anlage.

On that occasion I have also called attention to a very probable source of error in King's ('05)[1] experiments in view of which the evidence she brought forth for the independent development of the lens in *Rana palustris* in contradiction to Lewis's ('04) unmistakable evidence to the contrary, appears to be illusory.

Experiments of other observers (Bell '06 and '07 and Ekman '14) also yielded results which decidedly contradict the idea of the independent development of the lens. And a very beautiful demonstration of the "lentogenic reaction" of the epidermis to a stimulus from an eye fragment was recently furnished by Wachs ('14, p. 430 and Figs. 46, 47, 48 and 49).

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[1] Cf. the footnote on p. 221 of this paper.

This author transplanted in well-advanced larvæ (hind legs developed) of *Triton tæniatus*, in which the eye was at the time of operation as fully developed as in the adult, a piece of the iris from the eye of one larva into the otic capsule of another larva of the same age. As a result he could in one case observe that a strand of epidermal epithelium which, owing to the operation, had grown in from the edge of the wound to the otic capsule, has given rise to "ein rundes Lentoid . . . mit konzentrisch gelagerten Linsenfasern . . .."

From all that has been said so far we may conclude that *there exists no valid evidence for the possibility of the origin of the lens by self-differentiation.* On the contrary, *observation in the normal development of the eye, evidence from teratophthalmic cases and all experimental evidence point to the correctness of Herbst's ('01) and Spemann's ('01) conclusion that the lens of the vertebrate eye depends in its development and differentiation from ectodermal epithelium upon a specific, apparently chemical, stimulus from the optic vesicle.*

This stimulus might perhaps be in the nature of an enzyme action by contact. Spemann ('05, '12), who recognizes the dependence of the development of the lens upon this specific stimulus in some species, assumes the possibility of a specific secretion by the optic cup or rather by the retinal part of the latter, to which, as he believes, may also be due the "regeneration" of the lens from the iris of the fully developed amphibian eye. While this hypothesis appears to have received strong support from the beautiful experiments of his pupil Wachs (*l. c.*), it seems to me perhaps premature to speak of a secretion in this case. For, aside from other considerations, the fact ascertained by Wachs (*l. c.*) that the supposedly secreted substance is not conveyed by the blood of the animal to any other part of the body would seem sufficient to indicate that the retina, as Wachs himself concludes, has no such endocrine function. Nor is Wachs's assumption of a secretion that "bleibt auf das Auge und seine nächste Umgebung beschränkt" (p. 446) justified by the results of his numerous experiments.

There is, moreover, good reason to believe that the capacity for the "lentogenic stimulus" is not at all restricted to the retinal layers of the eye cup. For evidence is not lacking that the tapetum nigrum is also capable of inducing the development of a lens (or lentoid) when in contact with ectodermal epithelium. Thus, as pointed out above (p. 235), some of the "independent" lenses recorded by Mencl ('08) very clearly owe their origin to contact of ectoderm with tapetum nigrum fragments ("Pigmentbläschen," "Pigmentanhäufungen." "Pigmentmasse"). It was also mentioned that in a considerable number of *Fundulus* monsters in my possession "independent" lenses and lentoids can be demonstrated to be due to this "enzyme action" of the tapetum nigrum. This ability of the pigment layer of the optic cup is also suggested by some of Spemann's ('12) and Stockard's (*l. c.*) figures.

Whatever the nature of this action may be, whether or not we agree to regard it as an enzyme action, as I am inclined to do, it seems evident that all layers of the optic cup are capable of it. Even the iris epithelium which genetically is also a part of the optic cup may also be capable of this action.

3. The Origin of the Secondary Lens—in "Regeneration."[1]

Bearing in mind this apparent ability of the embryonic ectoderm for lens formation—by "enzyme action," it may perhaps no longer be difficult to account, at least theoretically, for its secondary formation—"regeneration"—from the iris after the exstirpation of the primary lens from the fully developed eye of the amphibian larva or even the adult.

An attempt in this direction has already been made by Wachs (*l. c.*) who accounts for this phenomenon as a reaction of the epithelium of the iris to the "secretion" of the retina. Although I find it difficult to accept the evidence for this secretion, I believe that Wachs's idea is correct in the main. The secondary formation of the lens—from the epithelium of the iris—is probably a response (reaction or complex of reactions) to a chemical stimulus just as much as its primary development from the supraocular epidermis of the early embryo.

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[1] I am not inclined to regard the formation of the lens from the epithelium of the iris as a case of true regeneration in the precise meaning of that term. *From the histogenetic point of view* we must say that *this matrix* (*the iris*) *gives rise to the lens for the first time* and thus it *generates*, but does *not re*generate, it. *Considering*, on the other hand, the *secondary lens in relation to the whole visual organ or to the entire body of the animal*, it is difficult to deny that *we are here dealing with* a case of what is commonly regarded as *regeneration* ("Ersatz", "Nachbildung", "Nachwachsen", "Wiederwachstum"). Without wishing, therefore, to decide on the fitness of the term in this connection, I am employing it only in quotation marks.

But, it may well be asked, if there is no such secretion as assumed by Wachs, what is the nature of the chemical stimulus and whence, from what source, or from what part of the eye does it issue? Two possibilities suggest themselves as an answer to this query. It may be imagined that, owing to the operation, the iris comes into temporary contact with the inner wall of the optic cup, from which it might thus receive the stimulus for the formation of a lens in the same manner as in the embryo the supraocular epidermis from the optic vesicle. Such contact due to a collapse of the optic cup[1] may result from the methods of operation employed by Wolff ('95) and the other experimenters (with the exception of Wachs). However, I am not inclined to give serious consideration to this possibility, as this contact of the pupillary edge of the iris is not necessary for the "regeneration" of a lens, for in Wachs's experiments the method of operation excludes it altogether. The experiments of the latter author, however, do not, as he believes, exclude another possibility which to me seems well worthy of careful consideration.

Wachs (*l. c.*, pp. 416–426) has raised the question whether the iris is capable of forming a lens without any outside stimulus ("aus sich heraus"). He was led to consider this possibility by the results of some of his experiments, in which a fragment of the iris, implanted into the posterior chamber of an eye deprived of its lens "regenerated" a lens, even if it did not heal on to the iris. This lens may even be better differentiated than the lens simultaneously "regenerated" by the latter.

To decide whether the fragment of iris in these cases formed a lens under the influence of a secretion from the retina or—"aus sich heraus," he transplanted (in a number of experiments) a fragment of the iris under the skin of the head. The result was negative, as the piece of iris disintegrated and was resorbed.

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[1] According to a statement by Müller ('96) no lens is formed by the iris after extraction of the primary lens, if the vitreous body is injured. Were this statement correct, no lens could arise from the iris owing to its contact with the optic cup by collapse of the latter. It is evident, however, that Müller's observation is incorrect, for in Wolff's (*l. c.*) and in some of Fischel's ('00 and '02) experiments in which a lens or lentoids did form from the iris, the vitreous body was undoubtedly injured, since the whole optic cup was damaged.

Wachs concluded from this result that the site of this transplantation was apparently unfavorable and he, therefore, in the next experiments transplanted a fragment of the iris into the otic labyrinth, after first removing a part of the latter. The following results were obtained:

In several cases where the fragment was "small," *i. e.*, consisted of iris only without retinal cells or with some very few of the latter, no lens-like structures were formed. In a number of instances, however, where the fragment was "larger," *i. e.*, containing more retinal cells, lentoids were formed (apparently from the iris), while in several other cases the transplanted fragment was transformed into a small eye with a lens, which latter Wachs considers as formed from the fragment of iris. However, in a number of instances (pp. 425, 426–428) where the transplanted fragment contained many retinal cells and was in good condition ("trotz guter Erhaltung") no lens-like structures were formed.

These results are, obviously, inconclusive. They certainly do not permit of Wachs's conclusion that the lentoids and lenses of the "positive" cases owe their origin to a stimulus from a secretion of the retinal cells (carried with the transplanted fragment of iris). For, why could not such effect of the retinal "secretion" be observed in the "negative" cases in spite of considerable retina? From these results I can read no definite, unmistakable, answer to the question which they were to answer.

For the following reasons, however, I should regard the query as a very pertinent one.

We know that the retina (Spemann, Lewis, Le Cron, Bell, Ekman, Werber and others) can furnish the stimulus for the formation of a lens from an epithelial derivative of the ectoderm (even epithelium of the mouth—Mencl, '08, and Werber, '16*c*) by contact with it. Besides being capable of furnishing the stimulus for this "lentogenic reaction" the retina (an ectodermal derivative) is according to Fischel's ('00, '02) and my (Werber, '16*c*) observations capable also of responding to such a stimulus—the lentoids of the retina. Whence does this stimulus issue in the case of the formation of the retinal lentoids? Considering the fact that the retinal cells can both furnish the "lentogenic" stimulus and respond to it, it would certainly seem not far fetched to assume that the stimulus for the formation of the lentoids by the cells of the retina issues from themselves. In this case the retinal cells react to their own "lentogenic enzyme." Both the conditions for furnishing the stimulus and the latent potency of responding to such a stimulus are known to be present in the retina. May not this double capacity underly also the formation of the lens from the iris?

To answer the latter question it would be necessary to find out whether the iris besides its known potency of forming a lens (Wolff, Müller, Fischel, Wachs, and others) is also capable of furnishing the "lentogenic stimulus," if in contact with epithelium (or some other ectodermal derivative). Unfortunately, however, the question cannot be answered definitely at the present time.

There exists only one experiment (by Wachs, *l. c.*, p. 430 and Figs. 46, 47, 48 and 49) which suggests this possibility, but the conditions in this experiment are not quite clear. A fragment of the iris was in this case (No. 39) transplanted into the cartilaginous capsule of the otic labyrinth. This fragment stimulated the formation of a lens (Fig. 49) from a strand of epithelium that had grown in from the edge of the wound. It is uncertain, however, whether in this case the "lentogenic" stimulus issued from the iris or perhaps from retinal cells contained in the transplanted fragment. For while on p. 429 Wachs states that this fragment was "ein Stück der oberen Iris" and that "nach 28 Tagen . . . war das Stück mit Goldpigment noch deutlich sichtbar," on p. 447 (in the summary) he refers to it as "ein Stück des Auges" and in the record (p. 423) this case (No. 39) is enumerated among those in which "ein Stück des oberen Augenteiles" was transplanted into the labyrinth. Wachs himself, however, does not state whether he assumes that in this case the regenerated epithelium formed the lens under the influence of retinal cells or of the iris.

There is no reason, however, why the latter possibility should be excluded. It is certainly not excluded by those experiments of Wachs in which a fragment of the eye cup containing both iris and retina (cf. Fig. 50) transplanted "dicht unter die Haut" has failed to stimulate lens-formation from the latter, but instead has so altered it that it gave the appearance "einer kleinen Cornea." For in these cases the failure to induce the lentogenic reaction might have to be attributed to the retinal as well to the iris-part of the transplanted fragment; it may, however, be due to the circumstance that the skin of the relatively old larvae was no longer capable of responding to a "lentogenic stimulus." The latter possibility may be implied from the (above-mentioned) case (pp. 237 and 241) in which owing to the operation an ingrowing strand of epithelium formed a lens under the influence of the transplanted fragment of the optic cup. For, as Wachs himself concludes, we are in that case (No. 39) dealing with "Abkömmlinge von Hautzellen, die, neugebildet und noch undifferenziert, offenbar die gleiche Fähigkeit der Linsenfaserbildung haben können, wie einst die junge Haut . . . " (*l. c.*, p. 430).

But while there is, as we see, no definitely known instance of lens-formation owing to a stimulus from the iris, there is, on the other hand, nothing that would contradict the assumption that the iris is capable of exerting such a stimulus on ectodermal derivatives. Moreover, the very fact that the other parts of the optic cup (retina and tapetum nigrum) can furnish the lentogenic stimulus would make it appear very probable that the iris which genetically is also a part of the optic cup (proper), is likewise possessed of the ability to induce the "lentogenic reaction."

Granting the correctness of this assumption (which eventually may be borne out by suitable experiments), we would in the case of the iris be confronted by the same conditions as in the case of the retina. It is known that the iris can form a lens, and if, as seems probable, it can also induce the formation of a lens from ectodermal epithelium, the assumption may be justified that in the formation of a lens from the pupillary edge of the iris, which is an ectodermal derivative, its cells respond to their own "lentogenic enzyme."

These conditions apparently obtaining in the formation of lentoids from the retina and very probably also in the "regeneration" (secondary formation) of the lens from the iris are in principle similar to the conditions obtaining in the primary (embryonic) formation of the lens, although the morphogenetic scheme of the latter cannot be fully applied to either the retinal lentoids or to the lens formed from the iris. The chief difference between the two modes of the morphogenesis of the lens is that while the origin of the "lentogenic stimulus" and the part responding to it are locally separate in the primary formation of the lens, they would—granting the correctness of our interpretation—coincide locally in the secondary formation of the lens from the iris or of a lentoid from the retina.

In order to fully understand the morphogenetic process underlying the "regeneration" of the lens from the iris (or of lentoids from the retina), it would also seem necessary to explain just how the "lentogenic stimulus," the latent capacity for which is probably possessed by the whole optic cup proper, is activated on the extraction of the primary lens. An answer to this query is, I believe, partly given by some of Fischel's ('02) experiments, which I shall review in the following.

The lens is so tightly enclosed by the iris that on its extraction slight injuries to the latter result. No matter how carefully the operation may be performed, slight, unnoticeable, lesions (abrasions?) of the cells of the pupillary edge of the iris may be unavoidable.[1] The significance of these, otherwise perhaps negligible, physical alterations of these cells of the iris resulting from the operation suggested itself to Fischel ('02, pp. 106–109 ff) from the observation of small lenses and lentoids formed not only by the pupillary edge but also from other parts of the iris. Being aware of accidental injuries to the eye cup and to the iris in some of his experiments he thought of a possible causal connection between the latter and such lenses or lentoids located in various parts of the iris. In order to test the correctness of his tentative interpretation he performed a number of experiments in which besides the exstirpation of the lens various parts of the iris were purposely injured. On microscopic examination of the results he was able to observe that small lenses or lentoids were formed from just such intentionally, and still discernibly distorted, parts of the iris. In several experiments Fischel observed besides the formation of a lens from the pupillary edge of the iris small double lenses, or twin (fused) lenses and in one case even three lenses in other parts of the latter. For the formation of these double (or multiple) structures Fischel, I think, correctly accounts by close proximity to each other of two (or more) places of injury.[1] Even the size of such lens-like structures he regards as dependent upon the extent (number of cells) of the injured area.

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[1] According to E. Uhlenhuth (quoted from Loeb '16) who cultivated fragments of the iris by Harrison's explantation method, it is to these abrasions that the loss of the pigment by the cells of the iris is due. Owing to this injury of the cells the pigment granules are liberated into the lumen of the optic cup where, as Wolff ('95) has observed, they may be absorbed by leucocytes.

The lens formed by the pupillary edge of the iris after exstirpation of the primary lens is usually as large and as well differentiated as the latter. This, according to Fischel, is due to the circumstance that on extraction of the lens a large area comes under the stimulus of the injury, and also to the fact that the pupillary margin of the iris being an epithelial fold, it affords the best means for the formation of a lens-bud (the "Knoten"—Wolff '95), the first step in which is always a folding of the corresponding part of the epithelium.

Another apparent confirmation of this "Reizhypothese" was furnished by the lentoids of the retina. The latter were observed by Fischel ('02) in many experiments in which owing to the extraction of the lens the eye bulb sustained accidental injuries. The experimental test—intentional injury of the retina—again gave positive results. The "retinal lentoids" could be demonstrated to be due to transformation of thus injured cells or groups of cells of the retina into such lens-like structures. The fact that only the latter and not fully differentiated lenses of large size can be formed by the retina on mechanical stimulation is, according to Fischel, accounted for by the small area of such stimulation and by the difficulty of forming a large fold.

This ability of the retinal cells to become transformed into lens-like structures Fischel regarded already in 1902 as no more surprising than, (as demonstrated by his experiments), that of any part of the iris, or of the epithelium of the skin in normal ontogeny. Already at that time he regarded it as a primary potency of all derivatives of the ectoderm. As the additional factor, however, necessary for this transformation of ectodermal derivatives he assumed an unknown stimulus which in the "regeneration" of the lens from the iris "*in jenen Alterationen zu suchen ist, welche das regenerierende Gewebe direkt durch den experimentellen Eingriff selbst erfährt* . . . von welchen die Zellen der Iris bei der Linsenextraktion betroffen werden" ('02, p. 106).

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[1] In some experiments of Wachs (*l. c.*) in which a fragment of the iris was introduced into the posterior eye chamber of another animal whose lens has been extracted, both the implanted fragment and the animal's own iris formed a lens. In some of these cases where the fragment coalesced with the iris, two more or less fused lenses were observed at the point of coalescence, one formed by the foreign fragment and one by the iris.

This unknown stimulus Fischel ('16) now regards as a chemical one from a secretion by the optic cup, or more specifically, from the retina which in conformance with Spemann and Wachs he also assumes. To the stimulus from this secretion he attributes the transformation of the supra-ocular epidermis into a lens in the embryo as well as the like transformation of the cells of any injured part of the optic cup (iris or retina).

While in the main I agree with Fischel by recognizing the necessity of an injury to that part of the iris or retina from which the secondary lens or lentoid arises, as proven, I am, as already stated, inclined to doubt the probability of a secretion by the eye cup as the additional factor involved in the stimulus for the formation of the secondary lens. Undoubtedly, with the present data at hand, it is difficult to deny that some chemical process is apparent in the morphogenesis of both the primary and the secondary lens. This process, however, may, as was pointed out in the preceding pages, be due to a substance (a "lentogenic enzyme") apparently contained by all parts of the eye cup already in the stage of the optic vesicle or even earlier. The activation, however, of this "enzyme" seems in the case of the "regeneration" of the lens to be in some way due to the injury of those cells of the iris which subsequently become transformed into lens fibers.

What would yet remain to be explained is the nature of the relation of the injury of the cells to the activation of their own "lentogenic enzyme." On this point, however, I should venture no opinion at the present time.

One apparent objection to our interpretation may yet be considered.

It is known from experiments by Wolff ('03) that if a part of the iris be removed by cutting (iridectomy) without removing the lens, the iris will regenerate but form no lens-like structures. Wachs (*l. c.*) attempted to account for this fact by the assumption of a secretion from the lens which would inhibit the formation of another lens. The results of some of his experiments, he believes, substantiate this conclusion. In these experiments the lens was removed and subsequently the lens of another animal of the same or a related species was introduced into the eye. In no case in which the foreign lens healed into the eye was a lens "regenerated." This result, however, permits of another interpretation, in which no resort is made to the improbable assumption of the "antisecretion" by the lens.

Indeed, Wachs's own ingenious experiments performed for the solution of this particular part of the lens-problem would seem to disprove rather than to prove the assumption of a secretion. For they show that the "regeneration" of a lens is inhibited only when the (smaller) lens implanted into the eye, heals into it, *i. e.*, comes into close contact with the iris ("so dass die verengerte Iris sich ihr ringsum dicht anschliesst . . . ," p. 404). If, however, such contact is not effected—"liegt jedoch die aus dem jüngeren Tiere implantierte kleinere Linse mehr oder weniger in der vorderen oder hinteren Kammer, so wird eine Regeneration eingeleitet."

Wachs has ascertained beyond doubt that the inhibition is in these cases due not only to the mechanical effect of the contact, but very apparently also to a chemical action. While, however, he strongly inclines to the belief that the chemical action is due to a secretion, I think it can easily be shown also in all other of his experiments by which the matter was tested, that this chemical action is conditioned by contact of the implanted lens with the iris.

These important experiments of Wachs, strengthened further by some interesting results of Fischel's ('02) experiments[1] would seem, however, to leave no doubt that the lens contains something, some substance that apparently inhibits the formation of another lens from the iris which would invariably occur in its absence.[1] This substance, however, again need not necessarily be secreted, in order to neutralize the action of the "lentogenic enzyme." Just what the nature of this substance may be, is, of course, altogether a matter of conjecture. It might perhaps be imagined as an "antibody" ("antigen") contained in the peripheral part (the epithelial cells) of the lens and the realization of its inhibiting action may be due to close contact of that part of the lens with the epithelium of the iris.

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[1] Fischel exstirpated the lens and replaced it by small spherical fragments of the potato tuber. Whenever this "imitation lens" was large enough to fit the pupilla, no "regeneration" of a lens from the latter took place. If, however, its diameter was smaller than that of the pupilla, partial "regeneration" of the lens or at least an "attempt" towards it was noted, complete "regeneration" being impossible owing to mechanical obstruction by the potato fragment. This experiment clearly demonstrates that the extraction of the lens eliminates not an indifferent body, but one with a specific, *inhibiting*, action.

This "antibody" being, just like the lens, a product of the "lentogenic reaction," we may perhaps in the case of the vertebrate eye eventually have a striking example of some of the factors (synthetic enzyme action—Loeb, '16) concerned not only with development and growth, but also with the limitation of the latter[2] for the attainment of proper size relations, and with the maintenance of a chemical equilibrium indispensible for the undisturbed existence of a structure or an organ.[3]

[1] Fischel ('16) has recently furnished another interesting example of the apparent chemical action of the lens which he also considers as a "secretion." He transplanted in larvae (about 3 cm. large) of *Salamandra maculosa* the exstirpated lens under the skin of various parts of the head or trunk. As a result he observed that, while the lens underwent a gradual dedifferentiation and eventual absorption, the skin above the transplantate showed remarkable changes. The unicellular glands (the cells of Leydig) disappeared from the corresponding region of the skin, which, owing to the morphological changes, by its appearance suggested a similarity to "frühe Entwicklungsstadien des Hautepithels" (p. 37). Eventually this region of the skin became transparent and was very similar to a fully differentiated cornea.—Similar observations were, as mentioned above (p. 242), recorded also by Wachs (*l. c.*) on transplanting parts of a fully differentiated optic cup under the skin.

[2] The fact that the "regenerating" lens does not grow indefinitely (as it might, if it were a benign tumor—regarded as such at one time by Fischel, '00), but is limited in its growth to attain just the size to fit the pupilla, has recently occasioned J. Loeb ('16) to raise the question regarding the factors limiting growth. According to our assumption it might perhaps be imagined that every tissue (or structure) elaborates during its development a substance (an "antibody") which inhibits its growth beyond certain limits. In this case both growth and its automatic limitation would be conditioned by the same factors of development.

[3] Fischel ('16) concludes from his experiments that the already fully differentiated lens needs the chemical influence of the eye for its normal (undisturbed) existence.

Osborn Zoölogical Laboratory,
Yale University, October 4, 1917.

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SEX-DETERMINATION AND BIOLOGY OF A PARASITIC WASP, HADROBRACON BREVICORNIS (WESMAEL).

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The problem of sex-determination is nowhere of greater interest than in the Hymenoptera. The occurrence of parthenogenesis as well as sexual reproduction adds interest to this matter, especially in view of the fact that considerable variation in conditions obtains, not only between the different families, but even between the minor groups of the same family.

The present studies deal with an insect, *Hadrobracon brevicornis* (Wesmael), belonging to the family Braconidæ.

The insects vary greatly in coloration, ranging from near black though various intermediate patterns to near yellow. In the near-yellow individuals black pigment usually persists in the antennæ, in the compound eyes, about the ocelli, and in small markings near the base of the wings. No experiments have been made to test the cause of color variation except that it has been shown that it does not yield to selection. It seems probable, therefore, that it is due to some environmental influence. It shows a certain correlation with size, for the dwarfed specimens are usually very dark while the larger individuals are either very light or of intermediate color. There is no apparent correlation of color with sex.

In view of the fact that genetic differences with respect to color exist in the honey-bee, I had expected the yellow and the black variations of *Hadrobracon* to reproduce themselves, but this is not the case.

Fig. 1, *A*, shows a light colored male and Fig. 1, *B*, shows a rather dark female. Sexual dimorphism is evident in the longer antennæ of the male and in the presence of the ovipositor of the female. Variation occurs in the number of antennal joints and in the general body size. The larger specimens are three to four millimeters in length, but minute individuals occur as well. The latter are probably starved. They are fertile, nevertheless, and give rise, when bred, to wasps of normal size.

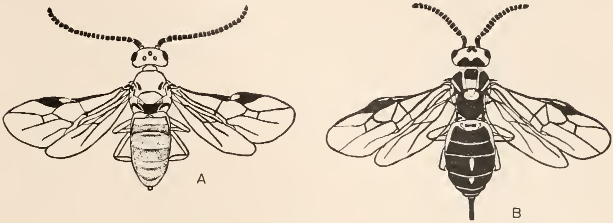

FIG. 1.

The insects mate readily as soon as they emerge from their white silken cocoons. The females deposit their eggs upon the bodies of full-grown caterpillars of the Mediterranean flour-moth, *Ephestia kuehniella* Zeller, and probably also upon other meal caterpillars. The caterpillars are stupefied by the sting of the wasps and remain quiet until the acephalous, apodous larvæ emerge and devour them. Several eggs are laid upon one caterpillar and as many as ten or a dozen wasps will sometimes develop. Usually the number is smaller. At a high temperature the length of a generation is ten days or less. The adult females may live as long as six weeks.

The work has been handicapped by lack of sufficient caterpillars at necessary times. As a consequence I have not been able to rear as many offspring as desired. The greatest number of progeny obtained from a single female was one hundred and twenty-eight, but I am convinced that many more could be secured if sufficient food were available. The technique of rearing and handling the moths is being developed and it is hoped that further work upon the wasps may be done as a result.

A protozoan parasite has been found in the caterpillars and in *Hadrobracon*. It has been eliminated by heat sterilization of the food and glassware. Its life history is being worked out by Dr. D. H. Wenrich. The small numbers in some of the families of *Hadrobracon* are due to the presence of this parasite.

My thanks are due to Dr. McClung for the facilities of the

Table I.

Inbred Progenies of Mated Females.

Designation of Parents.	Offspring.		
	Generation.	Sex.	
		♂.	♀.
♀ no. 1	F_1	49	47
F_1a	F_2	34	56
F_1b	F_2	26	29
F_1c	F_2	31	48
F_1d	F_2	31	47
Total..................	F_2	122	180
F_2a from F_1a	F_3	6	17
F_2b “ F_1a	F_3	3	9
F_2c “ F_1a	F_3	8	10
F_2t “ F_1a	F_3	2	10
Total..................	F_3 from F_1a	19	46
F_2d from F_1b	F_3	10	9
F_2q “ F_1b	F_3	2	8
F_2p “ F_1b	F_3	11	8
F_2o “ F_1b	F_3	21	13
F_2n “ F_1b	F_3	5	13
F_2k “ F_1b	F_3	15	18
Total..................	F_3 from F_1b	64	69
F_2e from F_1c	F_3	4	12
$F_2$1 “ F_1c	F_3	25	18
Total..................	F_3 from F_1c	29	30
F_2i from F_1d	F_3	2	6
F_2j “ F_1d	F_3	1	5
F_2m “ F_1d	F_3	13	13
Total..................	F_3 from F_1d	16	24
Total..................	F_3	128	179
$F_3$1 from F_2a	F_4	5	23
$F_3$2 “ F_2a	F_4	17	18
$F_3$3 “ F_2a	F_4	18	19
$F_3$4 “ F_2a	F_4	11	27
Total..................	F_4 from F_2a	51	87
$F_3$6 from F_2q	F_4	15	28
$F_3$7 “ F_2q	F_4	11	31
$F_3$8 “ F_2q	F_4	10	23
$F_3$10 “ F_2q	F_4	7	10
Total..................	F_4 from F_2q	43	92
F_3 12 ♀ from F_2t	F_4	21	39
Total..................	F_4	115	218

Table I.—*Continued.*

Designation of Parents.	Offspring.		
	Generation.	Sex.	
		♂.	♀.
F_4a	F_5	4	6
F_4b	F_6	8	22
F_4c	F_5	9	12
F_4g	F_5	4	3
F_4p ♀ from $F_3$6	F_5	19	19
F_4w ♀ “ $F_3$6	F_5	12	12
F_4y ♀ “ $F_3$8	F_5	26	25
$F_4$14	F_5	32	48
$F_4$15	F_5	7	19
Total..................	F_5	121	166
F_5a	F_6	14	9
F_5g	F_6	2	9
F_5m	F_6	5	6
Total..................	F_6	21	24
F_6g2	F_7	2	3
F_6g3	F_7	8	7
F_6k	F_7	2	4
F_6h	F_7	1	1
Total..................	F_7	13	15
F_7b	F_8	1	1
F_7c	F_8	8	9
F_7d	F_8	2	3
F_7e	F_8	0	1
Total..................	F_8	11	14
F_8a	F_9	4	2
F_8d	F_9	9	1
Total..................	F_9	13	3
	Total from 51 mated females	593 ♂♂	846 ♀♀

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In November, 1916, a female wasp (♀ No. 1) was found in cultures of the Mediterranean flour-moth at the Zoölogical Laboratory. A little later some cocoons of the wasp were found and these were isolated in vials. Both males and females emerged from the cocoons. The females were not mated but produced parthenogenetically families consisting entirely of males.

The first female (♀ No. 1), produced forty-nine males and forty-seven females. As later results show she had in all probability mated. Table I. shows the progeny of mated females descended from ♀ No. 1. No variation of sex-ratio correlated with age of the mother or lapse of time since mating could be detected.

The first column of Table I. gives the designation of the parents. The various matings of a given generation are designated by the letters of the alphabet, as F_3a, F_3b, etc. In case there are more than twenty-six matings in any generation, the succeeding matings are numbered, as F_31, F_32, etc.

Omission of letters or numbers denotes that the parents in question are elsewhere summarized or else produced no offspring.

The second column denotes the generation of the offspring and the third and fourth columns denote the males and females respectively.

The points of interest to be noted in Table I. are as follows:

Each of the fifty-one females produced females, and all but F_7e, which produced a single female only, produced males.

Considerable variation in sex ratio obtains in different fraternities, usually with excess or equality of females.

An attempt to correlate sex ratio in the maternal fraternities with that of the progeny resulted as follows:

Matings of progeny of F_1a (34 ♂♂ : 56 ♀♀) gave (19 ♂♂ : 46 ♀♀)
“ F_1c (31 ♂♂ : 48 ♀♀) “ (29 ♂♂ : 30 ♀♀)
“ F_1d (31 ♂♂ : 47 ♀♀) “ (16 ♂♂ : 24 ♀♀)
“ F_2a (6 ♂♂ : 17 ♀♀) “ (51 ♂♂ : 87 ♀♀)
“ F_2q (2 ♂♂ : 8 ♀♀) “ (43 ♂♂ : 92 ♀♀)
“ F_2t (2 ♂♂ : 10 ♀♀) “ (21 ♂♂ : 39 ♀♀)
“ F_36 (15 ♂♂ : 28 ♀♀) “ (31 ♂♂ : 31 ♀♀)
“ F_38 (10 ♂♂ : 23 ♀♀) “ (26 ♂♂ : 25 ♀♀)

Fraternities with excess females (131 ♂♂ : 237 ♀♀) gave 236 ♂♂ : 374 ♀♀ = 1 : 1.6.

Matings of progeny of ♀ no. 1 (49 ♂♂ : 47 ♀♀) gave 122 ♂♂ : 180 ♀♀
“ F_1b (26 ♂♂ : 29 ♀♀) “ 64 ♂♂ : 69 ♀♀

Fraternities with equality of sexes (75 ♂♂ : 76 ♀♀) gave 186 ♂♂ : 249 ♀♀ = 1 : 1.3.

It is seen that a higher female ratio is obtained in the progeny of those fraternities in which the females were in excess of the males than in the progeny of those fraternities in which the sexes were equal, but although this condition obtains the numbers are too small and the exceptions too numerous to be conclusive. Thus the progeny of F_1c, $F_3$6 and $F_3$8 had excess ot females but produced equality of sexes, while the progeny of ♀ No. 1 had equality of sexes but produced excess of females.

As mentioned earlier in the paper, virgin females gave only male offspring.

Twenty-six virgin females were isolated and produced 757 males. The fraternities consisted of 4 to 84 individuals. Eight females that were with males produced 146 males and no females. It is probable that they did not mate.

Table II.

Offspring of Females, Before and After Mating.

Designation of Parents.	Offspring.		
	Before Mating, ♂♂.	After Mating.	
		♂♂.	♀♀.
		to other than their own sons	
v ♀ 2	106	13	9
$F_3$11	7	5	2
F_5c	20	1	6
		to their own sons	
F_3f	7	5	7
F_4i	59	51	0
F_4n	16	11	27
F_4o	50	11	11
$F_4$1	30	0	2
F_5j	41	42	6
F_6a	40	2	2
Total from ten ♀♀	376 ♂♂	90 ♂♂ (excluding F_4i)	72 ♀♀

Total from thirty-six virgin females—1,133 ♂♂.

Total from sixty-one mated females (excluding those that were set with males but produced only males)—683 ♂♂, 918 ♀♀.

Table II. shows the progeny of ten females that were mated late in life after they had produced several males. Seven of these were mated to their own parthenogenetically produced sons. In every case only males were produced before the mother mated and nine of the mothers produced daughters after mating. Virgin female F_4i produced fifty-one males after being introduced to a male. It is probable then that she did not mate.

The excess of males (90 : 72) in the progeny of females mated late in life is probably due to the fact that they did not mate after being set with a male until after they had laid a few eggs.

Summarizing then we have sixty-one females set with males that produced both males and females (683 : 918), nine that produced only males (197) and thirty-six virgin females that produced only males (1133). *Not a single female has been produced from a virgin female.*

The conclusion might be drawn then that in *Hadrobracon brevicornis* fertilized eggs produce females and unfertilized eggs produce males. If this is comparable with conditions in the honey-bee and the hornet, it must be supposed that the male is a haplont; the female, a diplont. An alternative possibility would be that males are diplonts, in which case they might be formed either from unreduced eggs or from reduced eggs that have been fertilized by male-determining spermatozoa.

Cytological work is now in progress that confirms the theory that the male is haplont. The first spermatocyte division is abortive as in the honey-bee. Details will be published later.

Other work in regard to sex-determination in the Braconidæ is that of F. M. Webster (1909) and S. J. Hunter (1909) on *Lysiphlebus tritici*, belonging to the subfamily Aphidiinæ. The results of these investigations have been reviewed and discussed by A. F. Shull (1910). Both males and females were produced from mated females. Virgin females usually produced males but occasionally a few females.

The work done at the United States Parasite Laboratory at Melrose Highlands, Mass., indicates that males are usually produced from virgin females of Braconids and Ichneumonids, but Hemiteles, an Ichneumonid hyperparasite, produces females parthenogenetically as well.

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STUDIES IN THE SEX-RATIO IN MAN.

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The numerical proportion of the sexes has always been a subject of great interest. The question whether they occur with equal frequency in man bears a close relation to the problem of sex-determination and comparative sex mortality. While the original sex-ratio is conditioned upon sex-determination, mortality may in the course of time change it. It will be shown that sex-ratio is not constant but varies in the different periods of life and under manifold influences. Most of the information at hand in regard to sex-ratio is derived from statistics of new-born and adults, but as regards intra-uterine life as far back as conception, our knowledge on the subject is limited to a few conflicting estimates. One of the purposes of this paper is to elucidate as far as possible this last problem. Inasmuch as the supposition that sex is determined before or at the time of fertilization is generally accepted, one may speak of a sex-ratio of conceptions, which may also be called original, physiological, or primary sex-ratio. That at birth, therefore, should be termed secondary and finally the sex-ratio of adults, tertiary.

Tertiary Sex-Ratio.

The consideration of sex-ratio in adults will be limited to general remarks. With only a few exceptions European countries have a greater proportion of women than men. According to Rauber ('00) the sex-ratio[1] including all ages, for all Europe, is 97.8; for Asia 104.6; for Africa 103.3; for America 102.2, and for Australasia 114.3. According to Brooks ('87) the Australasian colonies had in 1881 a sex-ratio of 143.72 for the aborigines, and one of 118.64 for the population of foreign descent. However imperfect these computations may be, they nevertheless show that Europe, with its excess of females, assumes a unique position. The following statistics, taken from one of Rauber's tables, represent the number of females for every 1,000 males:

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[1] The most common method in use for representing the average sex-ratio is to determine a number which indicates the proportion of males to every hundred females. Unless otherwise stated, this is the method herein used. The sex-ratio is also frequently called *masculinity*.

Age in Years.	Austria, 1869.	England and Wales, 1871.	France, 1872.	Germany, 1875.
25–30	1,087	1,111	1,017	1,059
30–40	1,074	1,092	991	1,046
40–50	1,089	1,081	996	1,052
50–60	1,033	1,074	1,008	1,081
60–70	1,022	1,128	1,033	1,114
80–90	1,052	1,376	1,359	1,240

With advancing age the excess of females increases. This is still more clearly shown in a table by Prinzing ('05) taken from the census of 1900 in Germany:

Number of females for every 1,000 males:

0– 5 years	993	40–45 years	1,045
5–10 "	998	45–50 "	1,067
10–15 "	995	50–55 "	1,121
15–20 "	995	55–60 "	1,140
20–25 "	1,008	60–70 "	1,189
25–30 "	1,008	70–80 "	1,259
30–35 "	1,014	80–90 "	1,338
35–40 "	1,020	90– "	1,751

Inasmuch as in Europe sex-ratio at birth favors males, its subsequent decrease must be the result of a greater mortality among the latter, and in some countries, also, of a greater emigration of males. The unequal mortality is shown in a table by Ploss ('87). For every 100 females there occur the following numbers of male deaths:

Sweden	104	Spain	107
Russia (Europe)	105	Austria	108
Holland	105	Switzerland	108
Italy	106	Germany	109
England	107	Greece	111
France	107	Rumania	116

The sex-ratio of mortality may differ under various circumstances; for example, between the ages of 25 and 40 years according to Knöpfel ('07) in rural districts it is below, while in the urban population it is above 100.

SECONDARY SEX-RATIO.

The sex-ratio of the living-born is, on a rough average for all European countries, 105–106; but this number may vary markedly even in comprehensive statistics. Manifold explanations for these variations have been offered and these will be considered briefly farther on. In the individual countries small differences in the secondary sex-ratio may exist, as shown in the following examples taken from a table by Ploss:

Russian Poland	101	Denmark	105
England and Ireland	104	European Russia	105
France	105	Italy	106
Germany	105	Austria	106
Switzerland	105	Massachusetts	106
Belgium	105	Spain	107
Holland	105	Connecticut	110
Sweden	105		

Concerning countries outside of Europe there is little information. Newcomb ('04) states that in Japan the excess of males in more than a million births was practically the same as in European countries. According to the same author, it seems probable that among the negroes of the United States there is a slight excess of female births.

As a result of the unequal mortality of the sexes the secondary sex-ratio becomes reversed early in life. Kroon ('17) states that in Holland the sex-ratio of mortality during the first year of life is 119; that is, the mortality among male infants is one fifth again as great as among females. He also states that during the first two months of life this ratio reaches even 139. Analogous numbers for the first year of life are given by Prinzing ('06):

Italy	111	England	121
Rumania	115	Sweden	121
France	119	Denmark	121
Austria	119	Norway	123
Switzerland	120		

Kroon's statistics show that this high sex-ratio of mortality of the first year soon decreases, reaching its minimum—approximately 80—between the fourteenth and fifteenth year. According to Prinzing ('05) the mortality from the ages of 5 to 20 years is greater in females, and indeed in the latter part of this period it is the result chiefly of tuberculosis, for which disease the common occurrence of anæmic and chlorotic conditions at the time of puberty furnishes an excellent soil. After this age the sex-ratio of mortality increases rapidly and results in the reversion of an excess of males to an excess of females.

PRIMARY SEX-RATIO.

The sex-ratio of conceptions cannot be determined directly; however there is an indirect method of solving the problem of the original sex-ratio by means of computing the mortality of embryos and fœtuses. Only in case the sex-ratio of those dying in utero is equal to the secondary sex-ratio, will the primary be equal to the secondary. If male abortions were absolutely the same as female, then the primary sex-ratio would be smaller than the secondary. If the sex-ratio of mortality during pregnancy exceeds the sex-ratio of the livingborn, then the sex-ratio of conceptions will of necessity be greater than the secondary and indeed all the more so as the total mortality becomes relatively greater. It is necessary, therefore, to consider two factors in order to deduce the primary from the secondary sex-ratio. The following schematic representation will illustrate this:

1. How many male abortions and stillbirths occur to every 100 female abortions and stillbirths?

2. How many abortions both male and female, occur to every 100 living born?

100 living born with sex-ratio 105.5 + a stillbirths and abortions with sex-ratio b.

100 + a conceptions with sex-ratio x.

This scheme becomes complicated by differences in sex-ratio of mortality and in the relative rate of mortality in the various periods of pregnancy. The relative number of stillbirths differs to some extent according to the various authors. This is in part explained by the fact that the statistics are taken from various countries. Rauber states that the stillbirths amount to 4 per cent. of the annual 1,800,000 births in Germany. According to Carlberg ('86), the proportion of stillbirths to the total number of births in Livland lies between 2.58 and 2.90 per cent., while the percentage for Western Europe lies between 4 and 4.5 per cent. According to Prinzing ('07), reports show that from 1891 to 1900 in every hundred births there were the following proportions of stillbirths:

Austria	2.9	Holland	4.3
Switzerland	3.6	Belgium	4.5
Italy	3.9	France	4.6

These figures are somewhat increased when expressed in percentages of livingborn. Computing from Auerbach's ('12) statistics of over 100,000 births in Budapest, the stillbirths amounted to 3.3 per cent. of the livingborn. Bucura ('05) found that among 40,169 births in the Clinic Chrobak in Vienna 5.8 per cent. were stillborn; Le Maire ('06) found 5.7 per cent. among 40,339 births in Copenhagen. Both of these figures are too high, inasmuch as these authors did not use the term *stillborn* in the usual sense, a small number of abortions being included. Bernoulli accepts 4 to 5 per cent. stillbirths to the total number of births.

The relative number of abortions is extremely difficult to determine, inasmuch as everywhere large numbers, especially of the earlier months, remain unknown. Williams ('17) expresses himself on this point as follows: "A conservative estimate would indicate that about every fifth or sixth pregnancy in private practice ends in abortion, and the percentage would be increased considerably were the very early cases taken into account, in which there is profuse loss of blood following the retardation of the menstrual period for a few weeks." Other obstetricians give different estimates. Franz ('98) found 15.4 per cent. of pregnancies ending in abortion, Malins ('03) 19.23 per cent. Taussig ('10) estimates that one abortion occurs to every 2.3 labors, Pearson ('97) to every 2.5 labors. Auerbach reports that according to estimates for Berlin, abortions amount to one-sixth to one-tenth of the number of livingborn. According to the same author, there were in Budapest in 1901–05, 111,139 living born, and in the years from 1903–05, 7,702 abortions. Assuming an approximately equal number of livingborn for each year, the livingborn between 1903 and 1905 would amount to 66,678, of which number there would be 11.55 per cent. abortions. This percentage is doubtless too small;

Auerbach himself assumes that many abortions of the earlier months are concealed. The same author found that of his material 3,635 abortions, or almost half, fell within the first three months of pregnancy, and it is for this very period that the statistics are incomplete; therefore in reality much more than half the number of abortions belong to the first three months. According to Franz 42.6 per cent. and according to Dührssen even 59 per cent. of abortions occur in the third month alone.

The sex-ratio of stillborn is much higher than that of living-born. A table taken from Morgan ('13) gives the following sex-ratios of stillborn for European countries:

Norway	124.6	Denmark	132.0
Holland	127.1	Belgium	132.0
Germany	128.3	Austria	132.1
Hungary	130.0	Switzerland	135.0
Italy	131.1	Sweden	135.0

Such high sex-ratios of stillborn as that of Walter (260) or that of Tschuprow (400) are probably based upon relatively limited material and do not represent true ratios.

Auerbach gives detailed information concerning the sex-ratio of abortions. This author's material is distributed among the different months as follows:

7th	month	402 ♂	348 ♀	sex-ratio	116
6th	"	506 ♂	437 ♀	" "	116
5th	"	645 ♂	396 ♀	" "	163
4th	"	928 ♂	405 ♀	" "	229
4th–7th	"	2,481 ♂	1,586 ♀	" "	156.4

For the first three months of pregnancy he assumes that the sex-ratio of abortions is at least equal to that of the fourth month, namely 229. It seems to him more probable, however, that it increases in constant proportion; therefore he estimates 322 for the third month and 452 for the second month. According to Carvallo ('12) the sex-ratio of dead embryos up to the fourth month is 250; this figure he calculated from the statistics of Paris in 1908. Körösy ('98) found the sex-ratio of 3,781 abortions to be 152.4. Pinard and Magnan ('13) report on 1,229 abortions, the age of which is not stated. This material showed a sex-ratio of only 101.1. Rust ('02) also found the sex-ratio of 454 abortions from the first six months very low, namely 101.8. It is apparent how greatly these figures vary. A new contribution towards the knowledge of sex-ratio of abortions, even if based upon a relatively small amount of material is, therefore, not valueless, especially inasmuch as great care has been taken to determine age and sex. The material for these statistics comprises almost 600 fœtuses from the collection of the embryological department of the Carnegie Institution. Age classification was based upon the sitting height (Keibel and Mall '10). Rauber published the sex of 57 fœtuses with their sitting height; these have been made use of with the author's material. Normal and pathological fœtuses, for the most part white, were used in this study. In a limited number of cases no parental history was available; however, it is very probable that the majority of these also were white. Among the few specimens of races other than white, there is a preponderance of negro. The total material from three to ten months comprises 332 males and 315 females, showing a sex-ratio of 105.4. Rauber's material alone gives a sex-ratio of 159. The sex-ratio of the definitely white fœtuses amounts to 118.7. In the various months it is as follows:

Month.	Total.	White.
3d	123.7	108.3
4th	110.5	109.4
5th	108.1	163.4
6th	58.8	76.0

The material upon which are based the sex-ratios from the seventh to the tenth month is too limited to be of much value when represented for each month. The exceptional sex-ratio of the sixth month must be explained as a chance occurrence. The same may be true for the sex-ratio (78.9) of all the pathological fœtuses. According to the latter, female abortions are more frequently pathological, a finding which is difficult to correlate with the greater mortality of male fœtuses. The greatest deviations in the ratios obtained by Auerbach and Carvallo on the one hand, and by the author on the other, occur in the third and fourth months, for which Auerbach found the ratios to be 322 and 229, and Carvallo, 250. The author's corresponding figures are as low as 124 and 110. The great excess of male abortions in the early months of pregnancy, as found by Auerbach and Carvallo, may find its explanation in the fact that in the statistics used by them the sex of the fœtuses was determined by various individuals, who had not the necessary specialized knowledge. Early in the differentiation of the external genitalia only the expert can state the sex with certainty. At this time, and even later, the inexperienced, misled by the size of the clitoris as well as by other factors, may erroneously determine the fœtus as male. Fewer errors would be made if only those cases definitely male were reported as such, and all the doubtful cases were designated as female. Even granted that larger statistics might raise the sex-ratio, the latter would never reach the high figures stated by Auerbach and Carvallo and assumed by others. Just as sex-ratio of mortality following birth varies according to age, so is it found to be true for pregnancy. Nothing is known in regard to the sex-ratio during the first two months of pregnancy; however that of the third month might be used hypothetically for this period. The mortality of male fœtuses in the third month, which is about one fourth again as large as for females, is followed by a mortality in the fourth to the seventh months which is approximately relatively equal, for both sexes. During the eighth to tenth month this relation again shows a higher mortality for the males. The author's material from the fourth to the seventh month shows a sex-ratio of 101.1. However, on account of the great variability in the individual months, it is quite probable that of the male and female fœtuses that survive the third month, the number that perish during the following period up to the seventh month is relatively, not absolutely, equal.

If one proceeds to make use of the above citations and figures in computing the primary sex-ratio, rough and approximately average values must first be established. The following appear to be most probable:

For each		100 living born	with sex ratio	105.5
8th–10th	month	4 still born	" " "	130
4th–7th	"	9 abortions	" " "	106.3
0 3rd	"	14 abortions	" " "	125
		127 conceptions	" " "	x

From this, x or the primary sex-ratio is found to be *108.47*. The relative number of conceptions was estimated by Rauber to be 100 to 76 living born or calculated as above 131.6 to 100 living born. This number is somewhat larger than that obtained by the author, namely 127. The sex-ratio of conceptions was estimated by Bernoulli as 108.2. Slightly higher (108.7) is the ratio computed by Jendrassik ('11) from statistics collected by Bodio. Both these figures are strikingly similar to that of the author, namely 108.47. Lenhossék ('03) estimates the primary sex-ratio as 111; Auerbach as 116.4 but the latter believes that it would reach at least 125 if corrections were made.

Even if these approximate averages, which become constant only when based upon extensive material, must be accepted *cum grano salis*, it may nevertheless be stated with certainty that more males (probably not more than 10 per cent.) are conceived, and that at certain periods of pregnancy the relative mortality of males exceeds that of females by as much as one fourth.

Determination of and Changes in the Primary Sex-Ratio.

Now arises the question as to what determines this unequal distribution of sexes at conception. Its discussion dates back into antiquity, Hippocrates and Galen asserting that males originated from the right testis or the right ovary, females from the left. It has been proved that this theory is untenable; however, the idea of the possibility of two distinct varieties of spermatozoa was again revived in more recent times. Wilson ('05) distinguishes male-producing and female-producing spermatozoa. This might lead to an unequal distribution of sexes at conception. Morgan suggests that it may be due to a difference in the rate of travel of the two types of sperm, or that a disease process, or a factor such as alcoholism, might affect one type to a greater degree than the other. Hertwig ('12) attributes sex-determination to the ovum or the degree of its maturation, an advanced stage of maturation producing males. In this way he attempts to explain the difference in sex-ratio according to social class. Thury ('63) had proposed the idea that ova which are fertilized late may produce more males. Thus he explained the high sex-ratio among Jews, who, on religious grounds refrain from intercourse for seven days following menstruation. Lorenz ('98), Lenhossék and Orschansky ('03) are of the opinion that sex is subject to hereditary influences, inasmuch as they found families in which males predominated and those in which females appeared in excessive numbers. Newcomb, Woods ('06) and Heron ('06) deny this and show that inheritance plays no part in the sex-ratio. Numerous authors attribute its variations to the absolute and relative ages of the parents. According to Rosenfeld ('00) there is a decided preponderance of male children born to young and old fathers, as compared with those of middle age. Francke, from the statistics of Norway, found this to be true in respect to young fathers, but reached an opposite conclusion as regards old ones. Dumont ('94) found for Paris a sex-ratio of 101.9 when the fathers were from 18 to 25 years; 104.2 when the fathers were between 26 and 50 years and 97.5 when the fathers were over 51 years. According to E. Bidder ('78), the sex-ratio of births by mothers under 18 years and over 40 years is especially high. Sadler ('30) stated that the relative ages of the parents determines the sex-ratio; the latter is 86.5 when the father is younger than the mother, 94.8 when both are of equal age, and reaches 163.2 when the father's age exceeds the mother's by 16 or more years. Kollmann ('90) obtained an opposite result. He therefore draws the conclusion, based upon extensive material, that the sex-ratio is high when the father is younger, and low when he is older than the mother. At the same time he opposes the view that the absolute age of the mother has any influence whatever upon the sex-ratio. Stieda, on the basis of his investigations, reaches the conclusion that any influence of the absolute ages of the parents is out of the question, as he noted the highest sex-ratio when the parents were of equal age. Numerous other authors have occupied themselves with the question of parental age as an influence upon sex-ratio, but only two additional ones will be mentioned, Boudin ('62) and Stadler ('78). The conflicting views which have been presented suffice to show that nothing definite is known concerning a correlation between the age of parents and sex-determination; in fact such a correlation is hardly to be expected. Pearl ('08) in a very careful study demonstrated that there are more males produced when the parents are of different racial stocks. The well-known assertion that sex-ratio rises after wars, has evoked various attempts at explanation. The following few examples are given: Ploss ('58, '61) ascribes it to malnutrition of the mothers. Berner ('83) believes it to be due to the diminished concurrence which follows wars and which brings about an increased prosperity. Düsing sees the cause in the increased sexual demands upon the male, which also is said to increase the sex-ratio in polygamy. According to Newcomb, following the Civil War no increase in sex-ratio was observed. In the cases where a difference was confirmed it was so slight[1] as not to exceed the normal variations as shown by Lehr ('89), Carlberg and others, and is to be considered as such. Variations of sex-ratio have been determined not only for individual years and for groups of years, but also for the seasons. According to Goehlert ('89) in autumn and winter relatively few conceptions take place, but a higher percentage of these are male.

CHANGES IN THE SECONDARY SEX-RATIO.

The primary sex-ratio, as shown above, becomes transformed by an unequal intrauterine mortality of the two sexes into a different secondary sex-ratio. The greater mortality of males during certain periods of prenatal life is explained by Carvallo as follows: "les garçons sont plus fragiles." Auerbach also considers the male fœtus less resistant. Grassl ('12) finds an explanation for the difference in the viability of the germ plasma. Jendrassik speaks of hereditary reduction of vitality among the excess of males. Rauber explains the greater mortality of males by the greater demands of the larger fœtuses upon the mother, the latter not always being able to meet them; the production of a female does not require as much from the mother. Lillie ('17) offers the suggestion that the greater mortality among male fœtuses is a result of disturbance of the equilibrium that protects the male from the sex-hormones of the mother. It seems probable that this is the case, especially in the first part of pregnancy.

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[1] For example Henneberg's ('97) statistics show that in Holstein between 1835 and 1845 the sex-ratio was 105.76, after the period of war from 1846 to 1853 it was 106.67.

The excess of male stillbirths is ascribed by most authors to the more difficult labor attendant upon the greater size of the male, especially the circumference of the head. In regard to this question Dutton ('10) is of the opinion that at the time of birth the bones of the male skull are as a rule more firmly ossified than those of the female. In this connection he states also that with the advance of civilization the pelvic development in women is not proportionate to the cephalic development which is taking place in infants. This perhaps explains the fact shown by Bluhm ('12) that the relative number of induced premature births is on the increase. That labor in cases of male children more often demands artificial aid from the obstetrician is shown by Prinzing, according to whom 6.18 per cent. of male births in Württemberg called for operative measures, as compared with 4.67 per cent. in the births of female children. The process of labor itself is the cause of only a small percentage of stillbirths, the majority dying before labor sets in.[1] Therefore, the greater size of the male cannot in itself be held responsible for the high sex-ratio of stillborn. According to Treichler ('95) 29.6 per cent., and according to Prinzing ('07) 32.6 per cent. of all stillbirths are premature, and in the sex-ratio of these size plays but little part.

Frequently sex-ratio has been studied in relation to the pelvic diameters of the mother. The results are somewhat conflicting. Hoffmann ('87), Dohrn ('88) and Orschansky ('94) may be mentioned, according to whom the sex-ratio in children of mothers with narrow pelves is small; in contrast to this, Linden ('86) states it to be 133 in 360 births in which the mothers had narrow pelves. In case the size of the pelvis really has an influence, this can be exerted only upon the secondary sex-ratio in the way of elimination. In the same manner it seems evident that many of the factors which apparently affect the sex-ratio do not change it at conception, that is, they do not have any sex-determining effect, but by their influence upon intrauterine mortality they change only the sex-ratio of births. The well-known fact that the secondary sex-ratio among Jews is relatively high is explained by Düsing on the ground of incest, blood marriages being of frequent occurrence in that race. Schultze ('03), on the other hand, has demonstrated that inbreeding has no effect upon sex-determination. The relative infrequency of abortions and stillbirths among Jews, as has been shown, for instance, by Auerbach for Budapest, explains very simply the fact that the secondary sex-ratio among Jews is higher and therefore closer to the primary. Punnett ('03) and others have shown that the births among classes of lower social status present a lower sex-ratio than those of the rich. The explanation lies again in the fact that the greater frequency of abortions among women of the working classes who can spare themselves less during pregnancy and in whom pregnancies occur in more rapid succession[1] results in a greater reduction in the sex-ratio, which was originally equal in the two classes. In addition, this greater reduction of the primary sex-ratio in the poorer classes is due to the higher percentage of stillbirths; according to Conrad in Halle among laborers it was 5 per cent., while among upper classes it was only 2.1 per cent.; and according to Verrijn Stuart ('01) in Holland, among the poor 3.16 per cent. and among the rich 2.50 per cent. of all births. A further example illustrating how the primary sex-ratio was erroneously thought to be influenced is shown in its difference between legitimate and illegitimate children. Heape ('09) states that the sex-ratio of legitimate births among the white population of Cuba is 109.0, stillbirths included; that of the illegitimate only 105.95; there is even a greater difference among negroes, the sex-ratio being 97.91 for illegitimate children and 107.73 for legitimate ones. Heape immediately draws the conclusion that illegitimate unions result more often in the conception of females than do legitimate unions. According to Düsing the sex-ratio of legitimate births in Prussia, between the years of 1875 and 1887, was 106.37, that of illegitimate only 105.54; the stillborn in legitimate unions amounted to 3.91 per cent.; in illegitimate 5.32 per cent. A corresponding difference was demonstrated by Bertillon ('96) in the frequency of legitimate and illegitimate abortions. The greater mortality of illegitimate fœtuses reduced the sex-ratio to a greater degree. The rule that the sex-ratio is greater in legitimate than in illegitimate births is not, however, without exception. Srdinko ('07) found that the sex-ratio of legitimate births in Austria was lower than that of the illegitimate, and explains this by the fact that the illegitimate are for the most part Jewish, in which race abortions are less frequent. According to a number of authors, the sex-ratio of first born is greater than that of subsequent births.[1] It is especially high in older primiparæ, as shown by Ahlfeld ('72 and '76), Janke ('88) and Bidder ('93). Lewis ('06) reports that in Scotland the sex-ratio of first born was 105.4 and that of subsequent births 104.8. That this at least in part is also due to different intrauterine mortality may be supported by the following citations. According to Franz, abortions are more than twice as frequent in multiparæ than in primiparæ. Moreover, the first-born children are appreciably smaller than subsequent ones, as demonstrated by Schaetzel ('93), a condition which would suggest a lower rate of mortality before birth as a result of the relatively fewer demands made upon the mother (Rauber). Hansen ('13) states that in Denmark the first-born weigh on an average 3,457 grams; the second-born 3,607 grams, third-born 3,698 grams, the difference between the first and second being much greater than between the subsequent ones. Stillbirths, however, according to Duncan and Duke ('17) are more frequent among first-born than among second- and third-born, in spite of their smaller size; only in the case of children from the sixth pregnancy dies the percentage of still born exceed that of first-born. However, inasmuch as many more abortions than stillbirths occur, comparatively little importance can be attached to this.

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[1] According to Ladame ('04) those dying during labor amounted to 36.4 per cent. of all the stillborn in Switzerland in 1900.

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[1] According to Düsing ('84) the longer the intervals between births, the greater is the sex-ratio.

Besides the above mentioned causes for the variations found in sex-ratio, many others have been discussed in the literature. Only a few of these factors actually exert any influence upon the sex-ratio of conceptions. The changes have all been found in the secondary sex-ratio and the probability is great that the factors causing them play not a sex-determining but a sex-eliminating rôle. This is true chiefly in regard to changes resulting from locality, such as rural and urban districts, from age, nutrition and health[1] of the mother. To what extent race determines the sex-ratio of conceptions cannot as yet be stated; however it is not improbable that differences exist in various races.

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[1] A table demonstrating this may be consulted in Newcomb's article.

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[1] Schenk ('98) asserts that diabetic mothers bear more female than male children.

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BIOLOGICAL BULLETIN

SPERMATOGENESIS OF THE PACIFIC COAST EDIBLE CRAB, *CANCER MAGISTER* DANA.

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1. Introductory Remarks.

The Decapoda have always been of absorbing interest to the cytologist and considerable has been written concerning their germ cells. In a previous publication ('14), I have given a review of the important literature on the subject, and by referring to this article it becomes apparent that practically all the investigations have been confined to the male reproductive elements of the Macrura and Anomura. Virtually nothing has been done with the Brachyura; in fact there isn't a single complete spermatogenesis of a crab known. Carnoy ('85) observed and pictured a few of the early proliferation stages in the spermatogenesis of *Carcinus menas*, while Binford ('13) hastily described the spermatogenesis of the Atlantic coast edible crab, *Menippe mercenaria*. None of these investigators, however, have given details regarding the interesting processes of synapsis, reduction, chromosome numbers and the like.

During the past few years the writer has been gathering testicular material of numerous crabs which occur along Puget Sound, for the purpose of studying their spermatogenesis, and in the following pages the spermatogenesis of one of these forms, namely, *Cancer magister*, is described.

2. Material and Methods.

As already mentioned, the material from which these studies were made consisted of the testis of the common edible crab of the Pacific coast, *Cancer magister*. This Brachyuran is widely distributed along Puget Sound, and an abundance of material was available for study. Most of this was gathered in the vicinity of the Puget Sound Biological Station, Friday Harbor, Washington, during the summers of 1915 and 1916. In the latter part of June and the early portion of July, the testis of *Cancer magister* is in the best condition for the study of spermatogenesis. At these times every stage in the spermatogenesis process may be found within the male gonads.

The author worked almost exclusively with smears of the testicular cells. Sectioned material was also used for comparison and checking up results. The smears, however, were of greatest service and virtually all the deductions and illustrations were made from them. The manner in which the smears were prepared was as follows. Small pieces of the fresh testis were quickly mashed between two slides and immediately fixed in Bouin's fluid for about ten minutes. The slides were then washed in water until all traces of the yellowish picric acid were removed. They were next stained by the iron-alum haematoxylin method, with a counterstain of acid-fuchsin. Finally, they were run up in the usual manner through the alcohols into xylol and when fully cleared were mounted under cover-glasses with Canada balsam. Some of these preparations were unsurpassed for details regarding synapsis, chromosome numbers, spermatid transformations, and steps in the opening of the spermatozoa. With the exception of Figs. 1–15 and Figs. 67–69, all the drawings in the accompanying plates are from smear preparations. Figs. 1–15 are from sections, while Figs. 67–69 are from living spermatozoa as viewed in the crab's body fluids.

The material for sectioning was cut into small pieces and fixed with numerous fluids. The best fixatives, however, were found to be Flemming's strong and the Meves-Duesberg modified Flemming. The sections were cut 5μ in thickness and then stained by either the iron-alum hæmatoxylin method with a counterstain of acid-fuchsin or by the safranin, gentian-violet method.

The living spermatozoa were studied under the oil-immersion lenses in various fluids, such as the body fluids of the crab, sea water, in isotonic and hypotonic solutions of various salts ($NaCl$, KCl, $CaCl_2$, $NaNO_3$ and KNO_3), and in distilled water. By fixing the spermatozoa on the slide with either osmic acid fumes or Bouin's fluid, these structures could then be stained in iron-hæmatoxylin and acid-fuchsin and all the stages in their explosion could subsequently be studied.

3. Description of Testis.

The testis of *Cancer magister* has already been described elsewhere (Fasten, '15). It is a bilobed organ lying in the cephalothoracic region, below the cardiac chamber and above the digestive glands. Each lobe is profusely tubular and runs laterally along the stomach. During June and July these testicular lobes are prominently developed and fill up a large part of the upper cavity of the cephalothorax. Below the anterior portion of the heart the two lobes of the testis unite, and from this junction point two stoutly convoluted tubes, the vasa deferentia, originate and run posteriorly to the base of the fifth pair of walking legs where they open to the outside.

During the latter part of June and the early part of July the tubules in the outer and middle regions of each testicular lobe are usually undergoing rapid proliferation. Some of them are filled with spermatogonia, while others contain growth stages and still others show primary and secondary spermatocyte divisions. Also, many of the tubules show spermatids transforming into spermatozoa, although the mature spermatozoa are mainly confined to the tubules located near the inner or median portion of the testicular lobe as well as in the spermatophores of the vasa deferentia.

When sections of the tubules are examined, many of them are observed to be constructed on the same plan as those of *Menippe mercenaria* which were described and pictured by Binford ('13). Two or three well-defined zones may oftentimes be distinguished in one tubule. For instance, at one pole in the sectioned tubule there might be a thin layer of spermatogonia and the rest of the tubule might contain either transforming spermatids, or mature spermatozoa, or primary spermatocyte divisions, or those of the secondary spermatocyte stage, or even growth stages. When there are three zones in the tubule these are often made up of (1) a thin region of spermatogonia at one end, (2) a middle portion filled with transforming spermatids, and (3) a region of mature spermatozoa filling in the opposite pole.

The mature spermatozoa, when they enter the vasa deferentia, are surrounded by pouches known as spermatophores (Fig. 67). These spermatophores are formed by the secretions of the inner layer of epithelium which lines the vas deferent tube. For a more detailed description of these structures see Fasten, '17. During copulation the spermatophores are discharged from the vasa deferentia of the male and are deposited within the seminal receptacle of the female, where they remain dormant until the ova are mature for fertilization.

4. Spermatogenesis.

A. *Spermatogonial Stages.*

The spermatogonial cells generally line one end of the tubule. They are fairly large cells, with distinct cytoplasmic outlines and prominent nuclei. In *Cancer magister*, primary and secondary spermatogonial stages can be distinguished (Figs. 1–8). Of the two, the primary spermatogonium (Fig. 1) is somewhat larger in size, and from it by division (Figs. 2–7), is derived the secondary spermatogonium (Fig. 8).

The resting primary spermatogonium (Fig. 1) contains numerous large chromatin clumps within the nucleus. In some instances a number of linin strands could also be distinguished. The cytoplasm is more or less uniform throughout, but in a few cases large heavily staining masses, surrounded by clear outlines (Fig. 2), could be observed within it. A centrosome is almost always discernible.

When the cell begins to divide the large chromatin clumps of the nucleus undergo fragmentation (Fig. 2). This process continues until the chromatin becomes organized into a great number of heavily staining elliptical or oval structures distributed throughout the nucleus. The spermatogonium at this stage presents the picture shown in Fig. 3. Counts of these chromatin clumps were attempted, but they were found to vary considerably, ranging everywhere from forty to sixty-five. Binford ('13) has made similar observations in *Menippe mercenaria* and in the American crayfish *Cambarus virilis*, I ('14) have found the same sequence of events. Binford regards these chromatin clumps as the chromosomes which enter the equatorial plate of the spermatogonial metaphase. While these through a further fragmentation, undoubtedly form the ultimate number of chromosomes found during the metaphase stage of the spermatogonial division, yet I am inclined to the view that many of these chromatin clumps represent more than single chromosomes. This conclusion was arrived at after examination of numerous polar views of the chromosomes in metaphase plates of primary spermatogonia, in which over one hundred chromosomes could be counted. Furthermore, these chromosomes were smaller than the chromatin clumps shown in Fig. 3.

The nuclear wall surrounding the chromatin clumps soon breaks down and the cell enters the metaphase stage (Fig. 4). The chromosomes in the equatorial plate are dumb-bell shaped and the spindle fibers are rather delicate in appearance. The centrosomes can be easily distinguished at opposite poles. In polar views of sections of the equatorial plate, the chromosomes are observed to be distributed throughout the whole plane of the equator. They are small and spherical in appearance, and are so numerous that their exact number could not be determined. As already mentioned above, in many instances over one hundred of them could be counted. But on the assumption that there are twice the number of spermatogonial chromosomes as are found in the reduction division, there must be one hundred and twenty chromosomes in the spermatogonial stages of *Cancer magister.*

The anaphase (Fig. 5), and telephase (Figs. 6 and 7) stages follow each other quickly, thus dividing the cell into secondary spermatogonia (Fig. 8). These are somewhat smaller than the primary spermatogonia (compare Figs. 1 and 8), and in the resting condition (Fig. 8), the chromatin of their nuclei stains much more heavily. Otherwise, the secondary spermatogonia resemble the primary ones markedly and their division proceeds in exactly the same fashion. The ultimate divisions of the secondary spermatogonia produce the resting primary spermatocytes (Fig. 16).

In many spermatogonial strips of the tubules, some of the cells were found to be undergoing degeneration. In such cases, the cells lose their distinctness of outline and their nuclei come to lie in a syncitial mass of protoplasm. In many instances, the nuclei resemble those of the spermatogonia (compare Fig. 9 with Fig. 1); in others (Figs. 10–12), the nuclei become transformed into very large irregular structures with pseudopodia-like projections. These cells are the so-called "nutritive cells" and they may be best studied in tubules where mature spermatozoa are found. In *Cambarus virilis*, I ('14) have noticed similar cells.

The nutritive cells (Figs. 9–15) are very interesting structures. Their nuclei contain heavily staining chromatin masses, while the cytoplasm possesses numerous fat globules which stain intensely black with osmic acid. Many of the earlier investigators on the Decapoda such as Grobben ('78), Gilson ('86), and Herrmann ('90) have claimed that the spermatogonia are derived from the nutritive cells. On the other hand, St. George ('92), and Keppen ('06) claim the opposite, that the nutritive cells are derived from a transformation of the spermatogonia. This last-mentioned condition seems to be the case in *Cancer magister.*

When sections of the nutritive cells are examined, numerous stages like those seen in Figs. 13–15 may be observed, which strongly suggest amitosis. The fact is that the earlier workers on the Decapoda all claim such division in the nutritive cells. However, a careful study of the nuclei shows many of them to be amboeoid in appearance (Figs. 10–12), and this being the case, it is entirely possible to derive the stages represented in Figs. 13–15 from sections through such cells as are shown in Figs. 10–12. It is, therefore, very difficult as well as dangerous to come to any certain conclusions concerning amitosis from sectioned material.

B. *Primary Spermatocyte Stage.*

This stage follows the spermatogonial divisions. After a period of growth and synapsis, reduction occurs. During the growth period two definite spherical bodies, surrounded by clear spaces, make their appearance in the cytoplasm. These are the so-called chromatoid bodies, and they appear to be similar to the same structures which I ('14) have previously described in *Cambarus virilis.*

(*a*) *Growth Period.*—This period includes the preparatory stages, synapsis and tetrad formation. During the early prophases the chromatin in the nucleus of the resting primary spermatocyte (Fig. 16), consists first of a few large, heavily staining clumps, but these soon undergo a fragmentation process, whereby smaller chromatin masses (Fig. 17) are produced. In a few cells, linin threads which were rather indistinct and granular in appearance were observed. Sometimes large chromatoidal masses like those shown in Fig. 17 were seen within the cytoplasm.

The chromatin of the nucleus breaks up into still smaller structures and these then begin to weave out into thin leptotene threads (Fig. 18). At the same time the cell increases somewhat in size (compare Figs. 16 and 17 with Fig. 18). This stage, represented by Fig. 18, really marks the beginning of the growth period and from now on the increase in the size of the cells is more evident. When Fig. 18 is examined it can be seen that no continuous spireme is formed. The leptotene threads remain separate and may be distinguished from each other. Owing to the great number of these threads it was impossible to count them.

The primary spermatocytes now undergo synizesis. The leptotene threads migrate to one pole of the nucleus (Fig. 19), the so-called "synaptic pole" and arrange themselves in parallel pairs. This paired arrangement is very distinct and has been observed in a great many cells. While this is going on, two large round bodies, of more or less equal size, and surrounded by clear spaces (Fig. 19, *k*) make their appearance in the cytoplasm. These bodies were first observed in a smear preparation which was heavily stained. They stained exactly like the chromatin. In preparations which were strongly destained no trace of them was found. However, when these latter preparations were restained, the bodies loomed up with exceptional clearness. It is thus seen that although these structures stain like chromatin, yet they differ from chromatin in their affinity for nuclear dyes, and, in all probability, they are chemically different from chromatin. Wilson ('13), first described a similar body in *Pentatoma*, and called it a "chromatoid body." In *Cambarus virilis*, I ('14) have found a pair of chromatoid bodies appearing at about the same stage in the developing spermatocyte as in *Cancer magister*. After the leptotene stage, the chromatoid bodies of *Cancer magister* persist within the cells, and their subsequent history will be outlined when the respective stages of the maturation are dealt with.

Synapsis soon sets in. The parallel threads become more closely paired at the synaptic pole and the cell enters the pachytene stage, in which the pairs of leptotene threads fuse into thick gemini (Fig. 20). Here the union appears complete as no traces could be found of the longitudinal furrows which separated the original pairs of parallel threads.

After remaining fused for some time, the components of each geminus begin to unravel. Along every geminus a longitudinal split makes its appearance (Fig. 21), and simultaneously with this there is an opening of the paired threads at one end, while remaining attached at the other end, thereby producing figures which appear like 8, V, or less commonly like U (Figs. 21 and 22).

Another longitudinal split soon occurs along the arms of each geminus. In Fig. 22 the beginnings of this second longitudinal furrow are clearly visible in the two arms of the 8 which is located in about the center of the nucleus. By means of these two longitudinal cleavage planes, four chromatin threads are formed, distinctly opened up at one end and temporarily fused at the opposite end. The two pairs of these threads continue to diverge in opposite directions until they ultimately form X's (Fig. 23).

The pairs of threads on opposite sides of the central fusion point of each X now migrate closer to each other until they come to lie almost parallel. The central fusion point next disappears, and four thin threads arranged in two parallel rows are produced. A hasty glance at this arrangement makes it appear as if the geminus from which the four threads were derived, was traversed by a longitudnal and a transverse cleavage plane (see Fig. 23). However, prolonged and careful study reveals the true nature of the case, that the threads under discussion are the results of two longitudnal splits of each geminus.

The tetrads are soon formed. Each thin thread shortens and thickens into a spherical chromatin mass (Figs. 23 and 24), so that very soon every geminus is changed into four spherical chromosomes, representing a tetrad (Figs. 23–25). Each tetrad thus contains four univalent chromosomes. In the next step pairs of these univalent chromosomes fuse, resulting in two large bivalents attached to each other by linin strands. This condition is particularly well shown in Figs. 24 and 25. The condensation of the bivalents continues until they are changed into dumb-bell shaped structures (Fig. 25). The growth process is now completed and the cell is ready to undergo reduction.

It is quite evident from the above discussion that in *Cancer magister* we apparently have to deal with a case of parasynapsis, or side by side conjugation of the chromosomes. This conclusion was reached after prolonged observations upon numerous excellent smear preparations, which contained an abundance of synapsis material. The essential steps closely resemble those of *Cambarus virilis* (Fasten, '14).

(*b*) *Reduction Division.*—In the last stages of the growth period, the nuclear wall begins to break down (Fig. 24). The two centrosomes, which have been formed by a division of the original centrosome, migrate to opposite poles, and thin spindle fibers make their appearance between them and the chromosomes. The chromosomes are soon pulled to the equator of the cell to undergo reduction. In Fig. 25, the dumb-bell shaped bivalents are seen arranging themselves. Here the chromatoid bodies can also be clearly discerned at opposite poles. Each of these bodies is surrounded by its characteristic clear space and may be easily recognized from the ordinary chromosomes.

In the metaphase period (Fig. 26), all of the chromosomes are grouped in the equatorial plane and appear as large dumb-bells. When the component bivalents of each dumb-bell are examined, they are found to be large spherical bodies, about twice the size of the spermatogonial chromosomes. Furthermore, these bivalents do not show the equational furrows where the equational division of the second spermatocyte will take place. Binford ('13), in describing this stage in *Menippe mercenaria* claims that the chromosomes in the equatorial plate of one of his preparations were found to be ring-like in appearance. In other preparations which were destained to the degree of removing all the stain from the cytoplasm and the achromatic figure of the metaphase, this same investigator asserts that he could distinguish the individual four chromosomes of each tetrad. In *Cancer magister* no such results were obtained in spite of the fact that a great many smears and sections were carefully examined.

In polar views of the metaphase stage sixty chromosomes have been distinguished (Figs. 27 and 28). These are generally oval in shape; some of them being larger than others and they are distributed throughout the equatorial plane. Figures 27 and 28 are drawings of polar views as seen in smear preparations. Similar counts of polar views in sectioned material have corroborated this number for the reduction division.

The chromatoid bodies always migrate undivided to opposite poles of the cell. In many cases they occupy positions along the spindle fibers (Figs. 26, 29 and 30), while in other cases they may be seen in the cytoplasm (Figs. 31 and 32). When they occupy positions along the spindle fibers, one would be easily misled into regarding them as accessory chromosomes, especially so if no attention were paid to the various stages of the growth period. Wilson ('13) has cited numerous cases in which investigators have undoubtedly confused chromatoid-like bodies with accessory chromosomes. After citing these instances, Wilson, on p. 403, then makes the following significant remarks:

"Such facts make it clear that the presence of sex-chromosomes can not safely be inferred alone from the presence of chromosome-like bodies lagging on the spermatocyte-spindles, or lying near one pole. The presence of compact, deeply staining nucleoli during the growth-period is by itself equally indecisive. In some cases the 'plasmosome,' especially after certain fixatives such as Bouin's fluid, may stain quite as intensely as the chromosome-nucleoli with haematoxylin, safranin and other dyes (cf. Gutherz, '12). Decisive evidence regarding these bodies can only be obtained by tracing their individual history and by accurate correlation of the chromosome-numbers in the spermatogonial and spermatocyte-divisions. It hardly need be added that great caution is necessary in dealing with difficult material in which for any reason such a test cannot be completely carried out."

The anaphase stage (Fig. 29) follows upon the metaphase. The bivalents of the dumb-bells are separated and pulled to opposite poles. The chromatoid bodies also migrate in these directions. The division process continues and gradually the primary spermatocyte enters the telophase stage (Figs. 30 and 31). In the final telophase (Fig. 32), the chromosomes which have completely migrated to opposite poles become surrounded by thin nuclear walls. The cytoplasm has constricted off into two distinct portions and during this process the spindle fibers have also been constricted so that at their center a thickened "zwischenkörper" is formed. When this stage is completed two secondary spermatocytes are produced, each possessing one of the chromatoid bodies within the cytoplasm (Fig. 32).

C. *Secondary Spermatocyte Stage.*

The secondary spermatocytes formed during the ultimate telophase stage of the reduction division, undergo immediate transformations. No rest period could be determined. This is similar to what occurs in *Astacus fluviatilis* (Prowazek, '02), in *Menippe mercenaria* (Binford, '13), and in *Cambarus virilis* (Fasten, '14). The cells assume the metaphase stage (Fig. 33), and the chromosomes line up in the equator of the spindles in the form of dumb-bells. When polar views of these chromosomes are studied in preparations which have been greatly destained, sixty of them may be counted (Figs. 34 and 35). These chromosomes are about half the size of those found in the reduction division (compare Figs. 34 and 35 with Figs. 27 and 28). As for the chromatoid body, it generally lies at or near one pole of the cell, leaving the other pole without any such element.

The anaphase (Fig. 37) and telophase (Figs. 38 and 39) stages follow each other in logical sequence, resulting in the division of the secondary spermatocytes to form spermatids. Two types of spermatids (Fig. 40), are thus formed in equal numbers; one type which contains a single chromatoid body in the cytoplasm (Figs. 40 and 41), whereas, the second type is without this body (Figs. 40 and 43).

D. *Transformations of the Spermatids into Spermatozoa.*

The spermatids produced are, at first, small and their nuclei contain large masses of chromatin material which stain intensely with nuclear dyes (Fig. 40). The cytoplasm is homogeneous throughout and within it a rather prominent centrosome is found. In the second type of spermatid developed, the cytoplasm, in addition to containing the centrosome, also possesses the chromatoid body (Figs. 40 and 41).

The first noticeable changes undergone by the spermatids in transforming into spermatozoa, occur in the nucleus. The chromatin mass of the nucleus is gradually reduced to such a degree that it loses its intense staining qualities, and becomes quite homogeneous in consistency (Figs. 40–47). At first the large chromatin clumps break up into granular masses (Figs. 40, 43 and 44). Then these fragment still more completely until in the final stages there remain, respectively, three round chromatin bodies (Fig. 45); then two (Figs. 42 and 46), and ultimately one (Fig. 47). This remaining chromatin structure is spherical, stains intensely black with Heidenhain's haematoxylin and occupies the center of the nucleus. It may be said to be a nucleolus-like body which resembles a karyosome.

Both classes of spermatids produced undergo similar changes of the nucleus. In the second type of spermatid, however, an interesting change goes on in the cytoplasm, simultaneously with the transformations of the nucleus. Here the chromatoid body wanders from its position within the cytoplasm to the periphery of the cell and is soon expelled to the outside (Figs. 40–42), thus playing no further rôle in the transformations. From now on all the spermatids are exactly alike and they undergo the same modifications.

At about this time a densely staining mass makes its appearance in the cytoplasm (Figs. 46 and 47, *m*). This mass has been called a mitochondrial mass by Koltzoff ('06) and Binford ('13). It stains like the chromatin of the nucleus, and first makes its appearance at about the stage in the spermatid transformations where the chromatin of the nucleus becomes reduced to two clumps (Fig. 46). As to whether this mass consists of mitochondria or not is a debated question. The fact of the matter is that cytologists themselves are not clear as to which bodies within the cell are mitochondria and which are not (see Cowdry, '16). In the cells under consideration, no traces of mitochondria have been observed in the earlier stages of the maturation. This darkly-staining mass makes its appearance only after most of the chromatin within the spermatid nucleus has been much reduced. This would lead one to suspect that the mass might consist of chromatin which has diffused out of the nucleus and has accumulated within the cytoplasm (see Figs. 43–47). At any rate, this seems a likely probability.

The spermatids now reveal the following distinct structures, (1) a rather homogeneous nucleus (Fig. 47, *n*), with a karyosome-like body in the center, (2) a cytoplasm in which are found (3) a centrosome (Fig. 47, *c*), and (4) a mitochondria-like mass (Fig. 47, *m*), which stains like chromatin. These elements must be clearly kept in mind in order to follow up the later changes of the spermatids. In linking up these changes it is also necessary to exercise great care. Sectioned material, while helpful, is by itself wholly inadequate for this purpose. Smear preparations of the entire cells, on the other hand, give a true picture of what happens, and these were used almost entirely in the study of transformations.

The nucleus wanders to one pole of the spermatid (Figs. 48–50, *n*), while at the opposite pole a clear vacuole (Fig. 50, *v*), makes its appearance. Sometimes two clear openings (Figs. 48–49, *v*) may be seen, but these later flow together into a single one (Fig. 50, *v*). At the same time the mitochondrial mass wanders in between the nucleus and the vacuole (Figs. 48–52, *m*), and ultimately fills this entire space (Fig. 53). The centrosome increases somewhat in size and takes a position in the center of the mitochondrial mass (Figs. 50–53, *c*).

The mitochondrial mass now transforms into a ring resembling a doughnut, and the centrosome comes to occupy the center of its inner open space (Figs. 53 and 54, *c*). The upper portion of the nucleus also becomes located in this space (Figs. 54–58, *n*). At the same time, the karyosome-like body, situated within the center of the nucleus (Figs. 47–53) migrates upward to the middle of the upper portion of the nucleus (Fig. 54) until it comes to lie directly below the centrosome.

Binford ('13) in describing the transformation of the spermatids of *Menippe mercenaria* claims, on p. 156, that "after the mitochondrial ring is completed the nucleus becomes widely separated from it and the capsule (Figs. 50 to 52). This, however, is not always the case. In two preparations from which Figs. 33 to 35 and 37 to 43 were drawn, the nucleus remained fitted closely on the capsule as shown in Fig. 43. As the two different conditions were obtained with the same fixing fluid it is hardly probable that the difference was caused by the fixing."—In sections of testicular material of *Cancer magister*, a few of the transforming spermatids showed the conditions which Binford describes, but in smear preparations not a single such instance was discernible. I therefore suspect that Binford had to deal with defects which are often produced in cytological material which is prepared by the fixation and the sectioning methods.

Simultaneously with the last-mentioned changes, a second vacuole (Fig. 54, *v′*), makes its appearance in the anterior extremity of the original first (Fig. 54, *v*), or primary vacuole. At first this second vacuole looks like a small bubble of liquid which stains rather darkly with Heidenhain's haematoxylin. It soon increases in size, becomes more distinct (Figs. 54–57, *v′*), and stains somewhat lighter. It is quite evident that Binford's ('13) so-called "inner tubule" formed during the spermatid transformations of *Menippe mercenaria*, is the same structure which I have called the second vacuole in *Cancer magister*. My preparations, however, show no such stages which Binford describes and pictures (Figs. 52–60 of Binford, '13), for the development of this structure.

During this time the centrosome and the karyosome-like body of the nucleus unite (Figs. 54–56), and elongate into a rod-like structure (Figs. 57 and 58), the so-called central body (Figs. 56 and 57, *b*). At first the central body looks like a dumb-bell (Figs. 56 and 57, *b*), but as it lengthens out, it loses this appearance, becomes more rod-like (Figs. 58–60), and at the same time penetrates the inner or proximal portion of the second vacuole. While all these changes are going on the primary and secondary vacuoles are gradually transforming into primary and secondary vesicles (see Figs. 54–60, *v* and *v'*), and from now on they will be designated by the latter names.

At about the stage represented by Fig. 59, an opening makes its appearance in the middle of the outer, or distal end of the second vesicle. Simultaneously with this, the central body elongates still more (Fig. 59) and its outer extremity seems to hollow out into a thin tube (Figs. 60–62) which soon connects up with the distal opening in the secondary vesicle. As the outer end of the central body hollows out, a ring of densely staining material makes its appearance around the outer opening of the second vesicle (Figs. 61–63, *d*). In Heidenhain's haematoxylin this ring stains intensely black like the centrosome or the chromatin. This ring may be spoken of as a chromatin-ring and it becomes more distinct as the distal end of the central body hollows out (Figs. 61–64, *d*). Going hand in hand with these modifications are those which take place in the mitochondria-like ring and the nucleus. These two elements fuse into a single structure, a sort of nuclear-mitochondrial cup (Fig. 62, *h*), which stains quite homogeneously, although some part of the mitochondrial mass seems to persist around the wall of the first vesicle and stains more intensely than the rest of the cup. (see Fig. 62, *h*).

As the transformations continue, the second vesicle fits more compactly into the first vesicle (Figs. 63 and 64). The outer portion of the central body has completely changed into a thinwalled hollow tube, which stains lightly. The inner portion of the central body is solid, stains intensely with nuclear dyes and is embedded in the center of the nuclear-mitochondrial cup, immediately below the wall of the first vesicle. Figs. 63–65 show these structures nicely. Now the radial arms or rays of the spermatozoön (Figs. 63 and 64, *r*) make their appearance. They originate as outgrowths from the nuclear-mitochondrial cup, and in the finished state they are stout structures with pointed extremities (Figs. 63 and 64, *r*).

Soon the spermatids are completely transformed into mature spermatozoa, and in this state (Figs. 65 and 66) they look like ovoid, or spheroid structures, in which the radial rays are tightly coiled around the nuclear-mitochondrial cup. Figs. 65 and 66 are drawings of mature spermatozoa as viewed, respectively, from the side and bottom. An examination of these figures shows the central body (*b*) located in the middle, and surrounding it in order of sequence are the second vesicle (*v′*), the first vesicle (*v*) and the nuclear-mitochondrial cup (*h*). In Fig. 65, the structure of the central body (*b*) consisting of a hollow distal end and a solid proximal end, can be distinctly observed. In this figure may be also seen the densely staining chromatin-ring (*d*), located at the upper end of the secondary vesicle.

When the mature spermatozoa are studied in smear preparations which have been fixed with Bouin's fluid and stained by the iron-haematoxylin and acid-fuschin methods, then the second vesicle stains a dark amber color, while the primary vesicle takes almost no stain and remains clear. In sections fixed with Flemming's fluid and stained similarly, the second vesicle remains more or less transparent, while the first vesicle stains a dark brown.

The mature spermatozoa are at first free, but when they make their way into the vasa deferentia they are surrounded by the membranous pouches or spermatophores already mentioned under the discussion of the testis. Fig. 67 shows one of these spermatophores when it is first removed from the vas deferens of the living crab. Notice the numerous spermatozoa which are compactly stored within its interior.

5. Opening of the Mature Spermatozoa.

The mature spermatozoa within the spermatophores are dormant structures, but when they are removed from the spermatophores and placed in fluids whose concentration is less than sea water, they undergo interesting changes. In many ways these are similar to the changes which Binford ('13) describes for the spermatozoa of *Menippe mercenaria*.

The method employed in studying the opening of the mature spermatozoa of *Cancer magister* was similar to that employed by Binford ('13). Numerous spermatophores suspended in either crab's body fluids, or sea water, were placed on a slide and covered with a cover-glass. By applying pressure to the cover-glass many of the spermatophores were ruptured, thus liberating the spermatozoa. These could then be examined under the high powers of the microscope. By allowing numerous salt solutions, already mentioned under the section on 'Materials and Methods,' to diffuse under the cover-glass, all of the changes in the opening up process or the so-called explosion of the spermatozoa could be followed out quite accurately. Many of these spermatozoa in various stages of their explosion were fixed and stained on the slides and were then used for later study and comparison.

In the crab's fluids, in sea water, or in solutions of NaCl, KCl, $CaCl_2$, $NaNO_3$ and KNO_3 which are isotonic with sea water, almost no change occurs. Usually the rays of the nuclear-mitochondrial cup unravel, revealing spermatozoa which contain either three (Fig. 68) or four (Fig. 69) rays. In hypotonic solutions of the last-mentioned salts, the spermatozoa undergo an explosion, and change considerably in appearance. Osmotic pressure, undoubtedly, accounts for this explosion as was suggested by Koltzoff ('06).

The first step in the explosion of the spermatozoa is the extrusion of the second vesicle. This vesicle normally surrounds the central body and is embedded in the first vesicle (Figs. 70 and 71). When the second vesicle begins to extrude, it swells somewhat (Figs. 72–76, *v′*) in size and at the same time it stretches the upper portion of the first vesicle and makes it appear like a thickened ring (Figs. 72–76, *s*). Simultaneously with this, the hollow distal end of the central body which is also extruded, exerts a pull on its solid proximal portion, transforming it into a spine-like structure (Figs. 72–76, *b*), which stains intensely black with Heidenhain's hæmatoxylin. While these changes are going on, the nuclear-mitochondrial cup loses its rays and rounds out into a spherical body.

When the second vesicle has been completely extruded (Fig. 75, *v′*), then the first vesicle (Fig. 75, *v*) commences to evert and continues this process until it is completely turned inside out. These steps may be observed in Figs. 75–79, *v*. During the eversion, the darkly staining proximal end of the central body forces upward on its tubular distal portion until the latter is finally extruded completely to the outside (see Figs. 75–77, *b*).

The completely exploded spermatozoa present the appearances represented in Figs. 78 and 79. The upper portion consists of the second vesicle (*v′*), in the interior of which is contained the everted first vesicle (*v*), with its upwardly projecting spine-like body (*b*). The lower portion consists of the nucleo-mitochondrial cup (*h*), which has transformed into a more or less spherical structure. In many cases, stages like those shown in Fig. 80 were seen. These evidently are exploded spermatozoa in which the second vesicle has completely ruptured and disintegrated. Binford ('13) has observed similar conditions in *Menippe mercenaria*.

6. Discussion.

A. *Synapsis.*

During the last few years the parasynaptic view of chromosomal conjugation has been established in numerous species of animals. In 1900, Von Winniwarter first advocated parasynapsis amongst the mammals, but the view did not become firmly established until the Schreiners ('04, '05, '06, '07 and '08) published their important researches on the germ cells of many animals including mammals, birds, reptiles, amphibians, fish, molluscs and annelids. Of late the parsaynapsis view of Von Winniwarter and the Schreiners has been extended to a great many additional forms, and excellent reviews of the vast literature on this subject may be found in the recent publications of Montgomery ('11), Wilson ('12), Fasten ('14) and Wenrich ('16).

Among the Crustacea, parasynapsis has been established in some of the Copepoda (Lerat, '05; Matschek, '09; McClendon, '10; and Kornhouser, '15), and also in one of the Decapoda (Fasten, '14). In the decapod crustacean *Cambarus virilis*, I ('14) showed that during the growth period, the chromosomes conjugate in parasynaptic fashion, and as already pointed out elsewhere in this paper, this is the type of conjugation which occurs in *Cancer magister*. In both *Cancer magister* and *Cambaras virilis*, the great difficulty encountered in the study of synapsis was the immense number of chromosomes. However, after prolonged and careful study of the various stages in the growth period of these animals, one finds it rather difficult to interpret the conjugation of the chromosomes in any other way than by parasynapsis.

B. *The Chromatoid Bodies.*

Ever since Wilson ('13) called attention to a chromatoid body in the spermatogenesis of *Pentatoma*, similar structures have been described during the spermatogenesis stages of other forms. In *Cambarus virilis* (Fasten, '14), two such bodies were found which could be traced into the spermatid stages and then all traces of them were lost. In *Cancer magister*, a pair of chromatoid bodies make their appearance during the synizesis stage of the growth period, and during the reduction division these pass to opposite poles, so that the secondary spermatocytes each possess a chromatoid body. During the equation division, this body passes undivided to one pole, resulting in two types of spermatids, one type possessing a chromatoid body, while the other type is minus such a structure. It is also of interest that the chromatoid body is eventually expelled from the first type of spermatid thus playing no further part in spermatogenesis.

Concerning the nature and function of the chromatoid body, very little can be said. In only two forms, namely *Pentatoma*, (Wilson, '13) and in the decapod under consideration *Cancer magister*, has the full history of this structure been traced, and in both cases, it is expelled from the spermatids, thus appearing to play no definite rôle in the mature spermatozoa. Wilson ('13), in discussing the chromatoid body, makes the following trite remarks on page 402: "The nature of the chromatoid body thus remains problematical, but the facts are worthy of serious attention for another reason. Were the chromosomes very small, numerous, closely crowded, or otherwise unfavorable for exact study, and could not the entire history of the chromatoid body be so clearly traced, even an experienced observer might fall into the most confusing error concerning the relations of the chromosomes."

7. SUMMARY.

1.. During the latter part of June and the early part of July the testicular lobes of *Cancer magister* are in the best shape for the study of spermatogenesis.

2. Two spermatogonial divisions can be recognized, and these ultimately form the resting primary spermatocytes.

3. Sometimes, larger and more intensely staining cells are found interspersed among the spermatogonia. These are the nutritive cells, and it seems very probable that they have originated from a transformation of some of the spermatogonia. The nuclei of the nutritive cells are irregular in shape and many of them possess amœboid processes. In sections of some of the nutritive cells two or more nuclei are oftentimes found, and this might easily mislead one into concluding that amitosis occurs amongst them.

4. The resting primary spermatocyte undergoes a growth period, during which thin leptotene threads are produced through the fragmentation of the chromatin. No continuous spireme is formed as the leptotene threads appear distinct and separate.

5. During the growth period pairs of leptotene threads migrate to the synaptic pole of the cell, become arranged in parallel fashion and soon fuse parasynaptically.

6. During the synizesis stage of the growth period a pair of densely staining chromatoid bodies make their appearance in the cytoplasm. These are surrounded by clear areas and may have originated from some of the chromatoid masses found within the cytoplasm of some of the earlier stages in the spermatogenesis.

7. The first spermatocyte division is reductional. In the metaphase stage the chromosomes line up as dumb-bells, which are composed of pairs of bivalents. The chromatoid bodies pass undivided to opposite poles of the cell.

8. A polar view of the metaphase stage of the reduction division reveals sixty chromosomes distributed throughout the entire plane of the equator.

9. The division of the primary spermatocytes results in secondary spermatocytes, each of which contains a chromatoid body.

10. The second spermatocyte division is equational and immediately follows the reduction division. A polar view of the metaphase stage of the equational division reveals sixty chromosomes which are about half the size of those found during the reduction division.

11. The chromatoid body passes undivided to one pole during the division of the secondary spermatocyte, resulting in the formation of two classes of spermatids, one of which contains the chromatoid body, while the other is without such a structure.

12. The chromatoid body is soon expelled from the spermatids which contain it, thus making all the spermatids alike in structure and appearance.

13. The nucleus of the spermatid loses its large quantity of intensely staining chromatin, while at the same time a mitochondria-like mass makes its appearance in the cytoplasm. Also one or two vacuoles are formed in the cytoplasm.

14. As the transformations go on the nucleus becomes elliptical and wanders to one pole of the cell. The vacuoles fuse into a single large vacuole which then takes a position at the opposite pole of the cell. The mitochondria-like mass wanders in between these two structures, becomes ring-like, and within its center and above the karyosome-like body of the nucleus, the centrosome becomes stationed. Soon a second vacuole makes its appearance at the distal end of the first one.

15. The two vacuoles gradually transform into the first and second vesicles. The centrosome and karyosome-like body of the nucleus become fused into the central body, which runs through the middle of the second vesicle, while the nucleus and mitochondria-like ring unite into a nucleus-mitochondrial cup from which the rays of the spermatozoön are produced.

16. The mature spermatozoa are oval bodies tightly packed within membranous spermatophores.

17. When the mature spermatozoa are surrounded with salt

solutions possessing a lower osmotic pressure than either the crab's body fluids or sea water, they undergo an interesting explosion in which the vesicles and the central body are completely everted, while at the same time the nuclear-mitochondrial cup rounds out into a spherical structure.

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9. DESCRIPTION OF PLATES.

All the illustrations in the accompanying plates were made with the aid of the camera lucida. Figs. 1–66 and 70–80 were drawn under Zeiss apochromatic objective 2 mm. Hom. Im., Comps. ocular 12,[1] at a magnification of 2,720 times. Figs. 67–69 were drawn under achromatic lenses at an approximate magnification of 1,400 times.

ABBREVIATIONS.

b = central body.
c = centrosome.
d = chromatin ring.
h = nuclear-mitochondrial cup.
k = chromatoid bodies.
m = mitochondria-like mass.
n = nucleus.
r = rays or arms of spermatozoa.
v = first or primary vacuole (first or primary vesicle).
v′ = second or secondary vacuole (second or secondary vesicle).

EXPLANATION OF PLATE I.

FIG. 1. Resting primary spermatogonial stage.

FIG. 2. Spermatogonial prophase, showing fragmentation of chromatin. Within the cytoplasm two large, heavily staining masses, probably chromatoid masses can be distinguished.

FIG. 3. Late spermatogonial prophase. Note the compact chromatin clumps within the nucleus.

FIG. 4. Metaphase, primary spermatogonium.

FIG. 5. Anaphase, primary spermatogonium.

FIGS. 6 and 7. Telophase, primary spermatogonium.

FIG. 8. Resting secondary spermatogonial stage.

FIGS. 9 to 12. Nutritive cells. Observe the irregular nuclei and the fatty globules within the cytoplasm.

FIG. 13. Nutritive cell, in which the nucleus shows a constriction in the middle which appears to be suggestive of amitosis.

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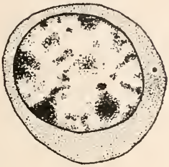
[1] I wish to express my indebtedness and gratitude to the Department of Zoölogy of the University of Wisconsin for the loan of a set of Zeiss apochromatic lenses (2 mm. oil immersion objective and compensating oculars 4, 8 and 12), which were of great service in the problem under consideration.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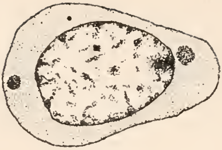
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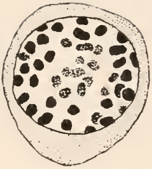
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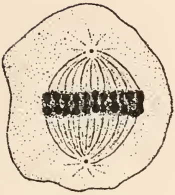
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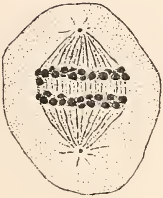
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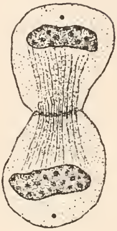
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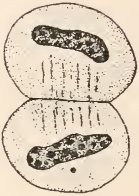
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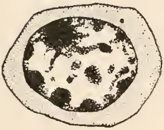
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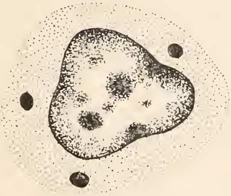
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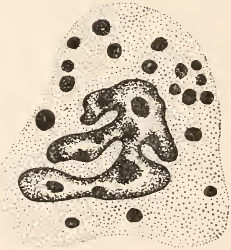
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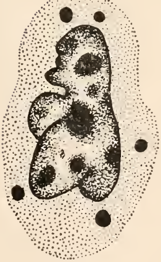
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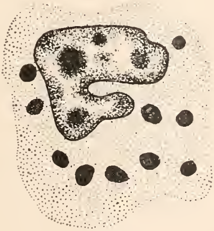
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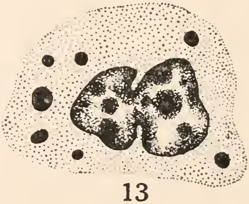
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EXPLANATION OF PLATE 2.

FIGS. 14 and 15. Nutritive cells, with two nuclei, suggestive of amitosis.

FIGS. 16 and 17. Early prophases, primary spermatocyte stage. In Fig. 17 two darkly staining chromatoid masses can be seen within the cytoplasm.

FIG. 18. Leptotene stage.

FIG. 19. Synizesis and synapsis stage. In this stage the leptotene threads have a paired parallel arrangement at the synaptic pole of the cell. The chromatoid bodies (*k*), and the centrosome (*c*), are also visible in the cytoplasm.

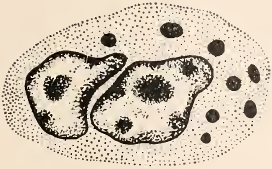
FIG. 20. Pachytene stage. The paired leptotene threads have united into thick gemini.

FIGS. 21 and 22. Diplotene stage.

FIG. 23. Postdiplotene stage.

FIG. 24. Tetrad formation. In this stage the nuclear wall begins to break down.

FIG. 25. Tetrads transformed into dumb-bells. The cell is entering the metaphase and the chromatoid bodies, surrounded by clear spaces are seen at opposite poles.



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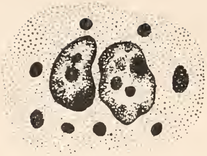

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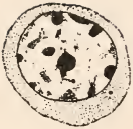

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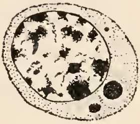

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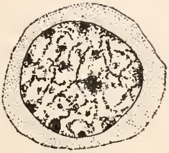

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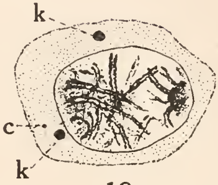

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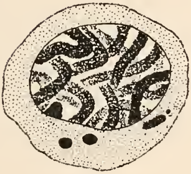

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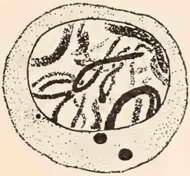

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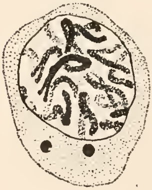

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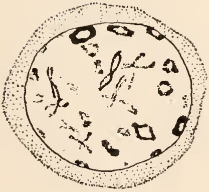

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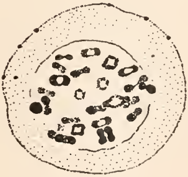

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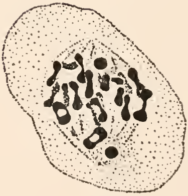

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Explanation of Plate 3.

Fig. 26. Metaphase, primary spermatocyte showing chromatoid bodies along the spindle fibers at opposite poles.

Figs. 27 and 28. Polar views, primary spermatocytes, showing 60 chromosomes.

Figs. 29 to 32. Anaphase and telophase stages of primary spermatocytes showing the chromatoid bodies at opposite poles. In Fig. 32 the chromatoid bodies are observed to remain in the cytoplasm.

Fig. 33. Metaphase, secondary spermatocyte, showing a single chromatoid body at one pole.

Figs. 34 and 35. Polar views, secondary spermatocytes, showing 60 chromosomes.

Figs. 36 and 37. Anaphase stages, secondary spermatocytes, showing different positions which the chromatoid body may occupy in the dividing cell.

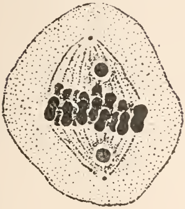
Figs. 38 and 39. Telophase stages, secondary spermatocytes. The single chromatoid body is at one pole.

Fig. 40. The two types of spermatids formed, one without the chromatoid body, and the other containing it. The centrosomes are the minute dark granules found in the cytoplasm of both these types of spermatids.

Fig. 41. Early stages in the transforming spermatid which contains the chromatoid body.

Fig. 42. Stage in which the chromatoid body is expelled from the spermatid that contained it.

Figs. 43 to 47. Early transformation stages of spermatids which are minus the chromatid body. Note the reduction of the chromatin and the appearance of the mitochrondria-like mass (*m*) in the cytoplasm. In Fig. 47 the single karyosome-like body occupying the center of the nucleus may be seen.



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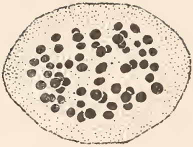

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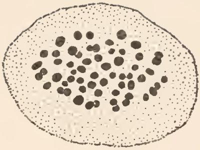

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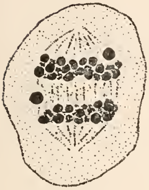

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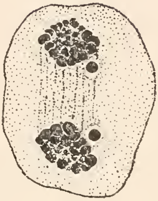

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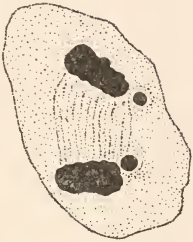

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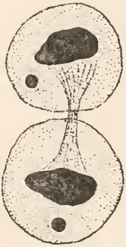

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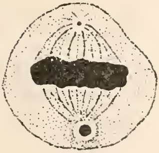

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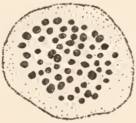

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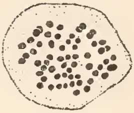

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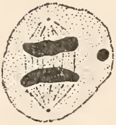

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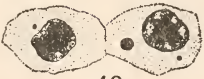

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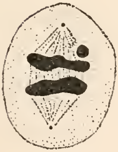

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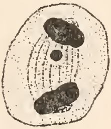

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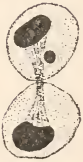

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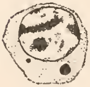

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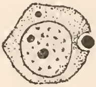

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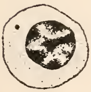

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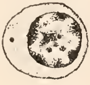

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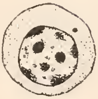

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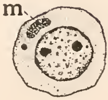

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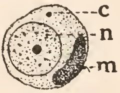

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N. FASTEN, DEL.

Explanation of Plate 4.

Figs. 48 to 53. Successive stages in the transformation of the spermatid, resulting in the primary vacuole (*v*) being formed at one pole, while the nucleus (*n*) occupies the opposite pole, and in between them the mitochrondria-like mass (*m*) and the centrosome (*c*) take their position.

Fig. 54. The secondary vacuole (*v′*) makes its appearance at the distal end of the first vacuole (*v*).

Figs. 55 to 58. Stages in the transformation of the spermatid in which the primary and secondary vacuoles transform into distinct vesicles, and the central body (*b*) assumes a dumb-bell appearance.

Figs. 59 to 62. Spermatid transformations showing the hollowing out of the distal end of the central body; the formation of the chromatin-ring (*d*), and the fusion of nucleus and mitochondria-like mass into a nuclear-mitochondrial cup (*h*).

Figs. 63 and 64. Formation of the rays (*r*) of the spermatozoön.

Figs. 65 and 66. Side and bottom view of mature spermatozoa, showing details of structure.

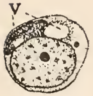
Fig. 67. Spermatophore, filled with mature spermatozoa.

Figs. 68 and 69. Three- and four-rayed types of spermatozoa, as seen suspended in the body fluid of *Cancer magister*.

Figs. 70 to 78. Successive stages in the explosion of the mature spermatozoön when surrounded with salt solutions of lower osmotic density than sea water.

Fig. 79. Spermatozoön which has exploded in distilled water.

Fig. 80. Exploded spermatozöon in which the secondary vesicle has completely disintegrated.



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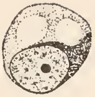

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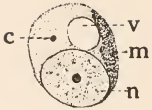

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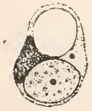

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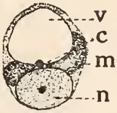

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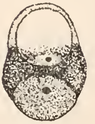

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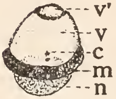

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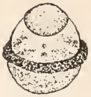

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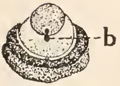

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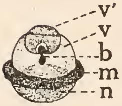

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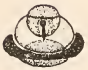

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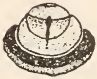

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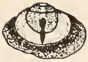

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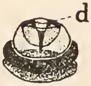

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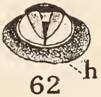

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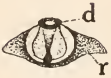

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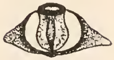

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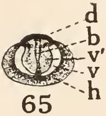

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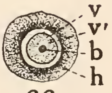

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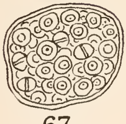

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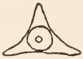

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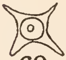

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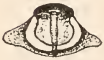

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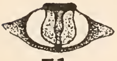

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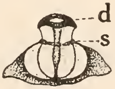

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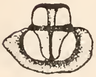

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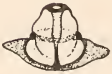

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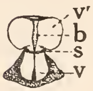

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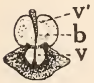

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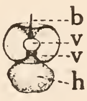

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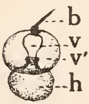

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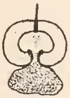

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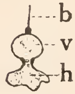

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N. FASTEN, DEL.

OBSERVATIONS ON THE STRUCTURE OF PROTOPLASM BY AID OF MICRODISSECTION.

WILLIAM SEIFRIZ.

TECHNIQUE.

The introduction of the Barber pipette holder brought into use an exceedingly ingenous technique which promises to open up a great field of research, in which not only many of the results got from fixed material can be confirmed or refuted, but in which observations on the structure and behavior of living protoplasm can be made with an accuracy and certainty not otherwise possible. This instrument, originally designed to hold miniature pipettes for isolation and injection work, is equally well adapted for the manipulation of glass dissection needles. Each needle is held in a three-movement clamp and extends into a small moist chamber placed on the stage of the microscope. The vertical needle-tips project up into a hanging drop in which the material to be dissected is suspended.

A description of this instrument and of the technique connected with it was first published by Barber (1914). Chambers (1915) has given a brief account of its use, and later (1917*c*) published a full description of the instrument and of the ways of making micro-needles.[1]

LITERATURE.

The literature published on microdissection studies is limited practically to the articles of G. L. Kite and Robert Chambers. Both of these men employed a Barber instrument essentially identical with that used by the writer. A list of their publications is appended to this paper.

TERMINOLOGY.

A serious difficulty encountered by the investigator in this field is the limited vocabulary which is at his disposal for the definition and description of the phenomena observed. We are little better off in choice of words than were the early microscopists whose knowledge of protoplasm was fully indicated by such adjectives as viscous, elastic, hyaline, etc.

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[1] It is of great importance in making sharp needles to have a minute flame. A very satisfactory micro-burner can be made—as was suggested to the writer by Dr. E. E. Free—from the smallest size (no. 27) hypodermic injection needle by snipping off the bevelled end with sharp shears.

In the possible range in consistency between the two extreme conditions of a colloid, *i. e.*, extreme liquidity and the solid state, the writer will distinguish ten degrees of viscosity, namely—watery, very liquid, liquid, slightly viscous, rather viscous, decidedly viscous, very viscous, extremely viscous, gel, and rigid gel—and has employed these expressions, for want of better ones, to describe the comparative viscosity of protoplasm. Their use will eliminate, in part, the vagueness from such general terms as liquid and viscous.

Protoplasm, which includes "all the living components of the cell-body" (Strasburger, 1891, p. 13), possesses many lifeless inclusions which materially affect its consistency, and they must be taken into consideration when the viscosity of protoplasm is discussed. As hyaloplasm is usually restricted to the hyaline border of non-granular plasma, the term matrix is used to indicate the translucent and more homogeneous fluid in which the various included granules are suspended.

Protoplasm, as such, frequently possesses a consistency different from that of its matrix. The latter may be watery yet the density of the protoplasm in toto markedly greater. It is, therefore, important to keep clear the distinction between the protoplasm as a whole and the matrix in which the various inclusions are imbedded.

MATERIAL.

The experimentation leading to the following results was carried on in the Harpswell Laboratory at South Harpswell, Maine. The writer wishes to thank the director, Dr. J. S. Kingsley, for the use of a room in the laboratory and for many other privileges enjoyed there.

The chief problem for the botanical microdissectionist is the obtaining of material delicate enough to permit dissection by fine glass needles. Even the wall of the frail alga *Spirogyra* can be entered only by the sharpest and most substantial needle that can be made. All in all, the most satisfactory objects for both zoölogical and botanical workers are the ova of marine organisms, although the myxomycetes are in some respects still better, for we have in them the largest masses of pure protoplasm known.

The observations here published were made upon myxomycetes, pollen-tubes, the oögonia, ova, and embryos of *Fucus*, and the ova of *Echinarachnius*.

OBSERVATIONS.

MYXOMYCETES.

Plasmodia of *Ceratiomyxa*, *Badhamia*, *Arcyria*, *Cribraria* and *Fuligo* were studied.

The protoplasmic density of myxomycetes is such that a needle traverses the plasma as through water, although it exhibits a slightly viscid property, for inclusions are pushed ahead of and to the side of a needle before they actually come in contact with it. However, if the point of a needle is broken off, making of it a minute pipette, cytoplasm and small inclusions rush into the opening with great rapidity and from quite a distance.

The vegetative plasmodium of a myxomycete is of very liquid consistency and remains so no matter how thin the film or filament of streaming plasma may be. If the plasmodium is not active a film or isolated globule of its protoplasm gels very rapidly. The consistency becomes so dense that a moving needle leaves a permanent furrow.

The old appellation "naked protoplasm," much used in reference to the slime-moulds, is in important respects a misleading one. The surface layer of a plasmodium is a definite morphological structure. The membrane is very extensile, slowly contractile, and surprisingly tenacious for so delicate a layer. This superficial layer can be isolated and held by one needle while stretched to several times its length by the other.

Within the plasmodium are definitely bounded smaller masses of protoplasm, which are apparently normal and in every respect identical with the surrounding plasma bulk. The origin of these smaller included protoplasmic masses was not observed, but their presence is common and they are readily distinguished from oil or other liquid inclusions. Several of these globules were isolated and dissected. Their limiting membrane is exceedingly sensitive, breaking before a perceptible indentation can be noted. Further dissection gave convincing evidence that this membrane is a morphologically differentiated layer. On breaking a globule some of the liquid protoplasm escaped. The pellicle of the ruptured mass stood out prominently and could be handled with a needle as one would handle a hair. It was of no appreciable thickness, yet quite rigid, though easily bent with no indication of being soft or glutinous. It had, therefore, during dissection, undergone a change—the normal, and extremely sensitive membrane had become a tough rigid gel. Such behavior well supports our interpretation of the plasmodium pellicle—not a surface-tension membrane nor a secreted wall, but a bounding layer of denser protoplasm. The plasmodial membrane is, then, a gel of appreciable thickness, tenacious, very extensile, contractile, and glutinous.

Next in importance to the possession of a definite morphological membrane is the capacity for forming one. The evidence which dissection work on myxomycetes presents on this phenomenon is, briefly, that the membrane is instantly and repeatedly reformed when ruptured by a needle, provided the protoplasm is normal. This is true whether the dissection be performed on a dry cover or in water; that is, the capacity is unchanged whether air or water is the surrounding medium. If gelation has set in the capacity for membrane formation is lost, although it may persist surprisingly long.

The living substance of slime-moulds is non-miscible in water.

POLLEN TUBES.

Of the many pollen grains experimented with those of the large blue flag (*Iris versicolor*) were the most satisfactory. The grains are large and germinate readily in almost any per cent. of sugar solution. The pollen of the beach pea (*Lathyrus maritimus*) is a fair substitute.

Repeated irritation of a pollen tube puts an end to protoplasmic streaming, although in some instances streaming may continue even after a tube has been punctured, and a large amount of its contents lost. Streaming, however, is not accelerated by irritation, nor is there any indication of a rush of protoplasm to wounded regions. The active protoplasm of a pollen tube is of very liquid consistency. On ejection it gels, though it does so slowly. Brownian movement sets in shortly after.

Ejected masses of protoplasm from pollen tubes develop membranes immediately on being freed. The capacity for membrane formation persists one to two minutes. Within three minutes the escaped protoplasm has become quite viscous. The membrane formed is surprisingly tough. Fragments of it can be dragged into the escaped plasma mass.[1]

Escaping protoplasm shows no sign of miscibility. Isolated groups of inclusions exhibiting Brownian movement, after ejection, were carefully observed and in every instance the cytoplasmic matrix was distinctly visible.

FUCUS.

The eggs of *Fucus* develop from divisions of the contents of oögonia which arise from single superficial cells of the wall of conceptacles that cover the fruiting branches. If these oögonia are teased out at a very early age they can be entered by a sharp needle. Very soon, however, the outer wall (exochiton) becomes too hard to be penetrated.

Oögonia.

In consistency the protoplasm of young uninucleate oögonia is very liquid. The wall is thick (2–4 microns), tough, and highly resilient. Slightly older oögonia, but still uninucleate, also possess very liquid protoplasm. Stages in development between young uninucleate oögonia and almost mature oögonia can not be observed, as the tough outer wall does not permit of dissection.

Immature Ova.

The contents of nearly mature oögonia can be squeezed out of the heavy exochiton by crushing the conceptacle with tweezers.

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[1] It was the intention of the writer to investigate the structure of the vacuole, of which so many are produced in the growing pollen tube. Freed vacuoles hold their shape even where exposed around the border of the plasma mass, apparently free of surrounding cytoplasm. When punctured the vacuoles collapse immediately. This behavior suggests that there is an enclosing membrane, and thus that the vacuole is apparently not really a vacuole but a sac. (De Vries, 1885, p. 467.)

Unripe eggs obtained in this manner are still held together by a jelly mass. The earliest stages so obtained were of oögonia in which division was complete but the eight eggs still of pentagonal outline in profile and closely appressed.

The protoplasm is of liquid consistency but shows marked signs of an increased viscosity over that of young oögonia. It tolerates a great amount of ill-treatment without showing any signs of injury. After half an hour of dissection there was no indication of gelation, the viscosity of the protoplasm remaining the same. Brownian movement, that unfailing criterion of degeneration, was not seen.

Slightly older oögonia in which the eggs, though still closely appressed, have rounded up somewhat, show further increase in protoplasmic consistency to the slightly viscous stage.

The separating membranes of these closely appressed masses of protoplasm are exceedingly delicate and of inappreciable thickness.

The capacity for membrane formation is complete. A remarkable property of the protoplasm of a young *Fucus* ovum is the rapidity with which it is enclosed by a wall after a needle has severed the egg. The very elastic glutinous membrane, ordinarily barely visible, is sufficiently pliable to be made of appreciable thickness when pressure is applied laterally with a tendency to compress.

The above described behavior of the ripening *Fucus* egg leaves no doubt that the plasma is a non-miscible fluid.

The stage of development of the eggs just described must be borne in mind. These ova, of slightly viscous consistency and enclosed by a delicate membrane, are nearing the completion of their development. They are still closely appressed within the oögonium, with several hours intervening before they would have been discharged as ripe eggs with gelatinous contents and a thick, hyaline wall.

The protoplasm of eggs of more advanced oögonia is of greater density, namely, rather viscous. It flows readily, but slowly.

The last stage in the development of the eggs before their discharge is especially noticeable because of an increase in thickness of wall. The enclosing layer is now one half of a micron thick. Notwithstanding the possession of a heavier wall these eggs can be dissected with as much ease, ending in the same results as those less mature ova having but a thin pellicle as covering. Droplets can be pinched off, the eggs torn from within outwards, or rapidly severed by a needle, without any indication of escaping protoplasm. The severed parts in every case instantly round up into droplets with walls apparently identical with the parent wall. This capacity to form a wall instantly at a ruptured point through conversion of the cytoplasm into a semi-rigid gel is not influenced by relatively great changes in the concentration of the surrounding medium, for it is as pronounced in very saline water as in normal sea-water.

There is no evidence of miscibility on the part of the protoplasm.

Fully Mature Ova.

The protoplasm of mature, normally discharged eggs is decidedly viscous, noticeably more so than that of well-developed unripe eggs. Thus has the highest degree of viscosity been reached in the development of the ovum, the transition taking place during the last periods of growth.

The wall likewise has undergone a marked change and become a hyaline, rigid gel, 0.8–1.2 microns thick, still very pliable and extensile, slightly contractile and exceedingly adhesive.

The wall of the mature normal *Fucus* ovum is capable of constant repair and this capacity often persists to the very last in a dying egg. Not until gelation of the protoplasm is well advanced does a rupture of the wall fail to be closed by a rapid conversion of the plasma matrix into a rigid gel. The capacity for wall formation is, then, one of the last essential properties of the living substance to be lost.

In all stages of its development before its normal discharge the egg shows no injury in consequence of dissection. The mature egg, on the contrary, is very sensitive to dissection, although its behavior is extremely variable. Every precaution was taken to prevent a misinterpretation of results due to observation of degenerated protoplasm. Such precaution was found to be more necessary with the ova of plants than with those of animals. To determine that the ripe eggs used for dissection were normal, part of each lot used was kept and tested for fertility.

The response to dissection of ripe unfertilized ova may vary from immediate disintegration to the toleration of two severances. Many eggs are very easily pinched in half by two needles rapidly approaching from opposite sides. The halves frequently round up into perfect spheres which can be again severed to form smaller protoplasmic droplets. While some eggs will show but slight if any increase in viscosity after many minutes of slow movement of the needles, others will suffer little or no dissection before gelating or completely disintegrating. Mere puncturing of the egg will often cause gelation. Thus does the ripe egg exhibit in one instance great sensitiveness and in another apparent indifference to stimulus.

This variability is carried even to parts of the same egg. One half of a severed ovum may gelate immediately without forming a wall over the torn surface, while the other half tolerates still another severance before gelating and thus losing the capacity for wall-formation. The presence of the nucleus in one half of the egg may be responsible for this difference in behavior (Townsend, 1897), although frequently both halves develop enclosing walls.

The protoplasm of the living ovum is at all times non-miscible in sea-water. This is to be expected if the capacity for wall-formation persists as long as the protoplasm is alive. In no instance of eggs examined in the various stages of ripening, while mature, and in the brief period following fertilization when the wall was still penetrable, was the plasma found to pour out and mix with the surrounding water.

The Unicellular Embryo.

Fertilization in *Fucus* is readily accomplished if active sperm are placed on a slide with mature ova.

Half an hour after application of the sperm the protoplasm of the fertilized ovum is found to be still quite viscous. A little later it becomes more liquid, for it readily oozes out of a puncture. Further development of the unicellular embryo shows a continued decrease in protoplasmic density.

The wall immediately after fertilization shows no increase in thickness and is still very pliable, extensile, and contractile. A little later it stiffens up until no longer elastic, though slightly resilient and pliable in so far that permanent indentations can be made. It can not now be severed by two approaching needles. In those few instances where the tough wall was punctured, and the protoplasm forced out, the ejected mass immediately developed a membrane. This plasma membrane is exceedingly thin, and quite elastic. It is interesting to note that the freed protoplasm here forms a delicate pellicle—not the tough wall of the one-celled embryo from which it came, nor the substantial though glutinous egg-wall which it would have developed in repairing a tear before fertilization.

At all times, from the moment of fertilization until dissection is no longer possible, the embryonic protoplasm is non-miscible. The ejected plasma shows no tendency to mix with the surrounding sea-water until after gelating, *i. e.*, after death.

The Multicellular Embryo.

As the two-celled embryo stage is neared the former egg wall has lost all of its former elasticity and glutinosity. It behaves under pressure like a ball of thin celluloid, resisting light pressure and returning to its former shape, unless forced beyond a certain point, when depressions made are retained. The two-celled embryo is punctured with great difficulty. The wall of the four-celled embryo is a very substantial affair, two microns in thickness, highly resilient and impenetrable to the sharpest needle.

The Degenerate Egg.

The behavior of the over-ripe ovum is extremely interesting and instructive in its bearing on the mechanism of the egg contents. As already stated, an ovum ultimately gelates and usually disintegrates. A gelated egg may be torn apart with great ease. If hardening has progressed far enough the egg may be cut in pieces as one would cut butter. If, on the other hand, the contents are not too solid the matrix may be readily drawn out into a long invisible thread. It is this gelated cytoplasmic matrix which is water-miscible.

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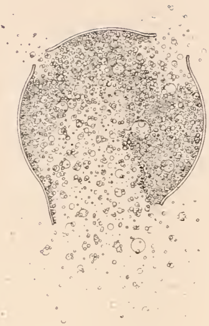

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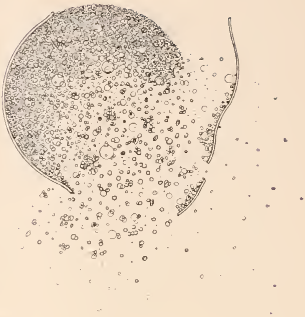

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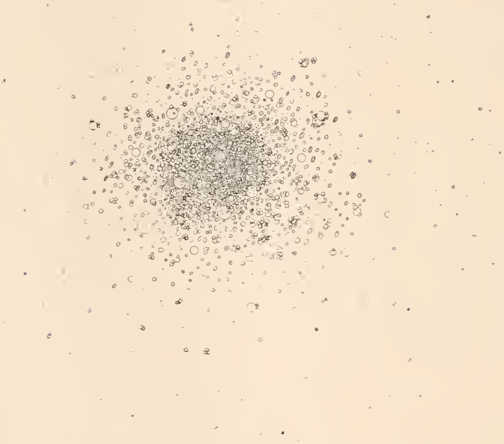


Description of Drawings.

Fig. 1. A disintegrating *Fucus* ovum in which smaller craters have burst forth subsequently to several seconds of activity on the part of the larger crater.

Fig. 2. A degenerate *Fucus* ovum in which discarded fragments of the wall are seen, and in which dissemination of granules is very active in one region while non-existent in another.

Fig. 3. The wild and rapid scattering of the inclusions of an exploded *Fucus* egg.

The disintegration of a degenerate egg is accomplished by the matrix going into solution with the sea-water. This may take place with such rapidity as to suggest a miniature explosion of the egg; or may be delayed and then it usually proceeds slowly, either in spasmodic periods of dissolution, or continuously until the entire granular contents has been disseminated. Often this is never completed. Where the disintegration is instantaneous (Fig. 3) it takes place immediately on the over-ripe egg coming in contact with a needle, or by the egg itself before actual dissection can be got under way. The miscibility of the matrix may be general (Fig. 3), or localized (Fig. 1). Where dissolution takes place without dissection some internal pressure, evidently osmotic, brings about a rupture. But it is difficult to see why such a force does not greatly expand so pliable a wall before breaking it. This is not the case. Furthermore, where disintegration has been instantaneous wall fragments are not to be found. When the process is gradual bits of it are only occasionally seen (Fig. 2). There is some reason for believing that the pliable, glutinous wall itself undergoes a change during degeneration of the protoplasm. If it does not go into solution it probably becomes a soft, inelastic gel.

A distributed, internal, osmotic pressure would be relieved once the egg-wall had broken at any one point, and one would not expect further eruptions. Quite the contrary is true. There may be a rather general dissemination of granules from one region for several seconds, time enough to relieve any pressure within, and subsequently, other craters break forth as shown in Fig. 1. These are localized centers of activity. That scattered regions of the egg contents are in different physiological states is further evident from the fact that certain localities may from the very outset remain inactive, never taking part in the general dissolution of the protoplasm, such as the exposed upper surface in Fig. 2.

The force responsible for a wild scattering of the inclusions in an ovum is a surface-tension one, due to an extraordinarily rapid miscibility of the degenerate matrix in the surrounding medium of sea-water.

ECHINARACHNIUS.

Microdissection work on the egg of the sand-dollar was undertaken for the purpose of comparison with the behavior of the plant ovum.

The difference in structure and behavior of *Fucus* and *Echinarachnius* ova before fertilization is not great. With fertilization two striking dissimilarities originate. There is in *Echinarachnius* an increase in viscosity of the protoplasm after fertilization. The path of a needle closes very slowly. Furthermore, there is very little change in the character of the egg wall. The wall of the young embryo is of no greater thickness than that of the ovum and but slightly more resistant.

The *Echinarachnius* egg occasionally tolerates a very great amount of dissection before completely deteriorating. At times, the capacity for wall formation persists in a region of still liquid plasma even after other regions of the egg have become an exceedingly viscous mass. This illustrates further the great diversity in the behavior of the protoplasm of eggs existing under apparently identical environments, and the marked difference in physiological condition of different regions of the same cell.

During the dissection of more than 20 eggs of the sand-dollar but one instance of miscibility was observed, and this was the rapid dissemination of granules due to the going into solution of the matrix of a gelated, that is, a degenerating ovum.

DISCUSSION.

In spite of the variety of objects studied and the differences in behavior of very similar material there are certain definite properties which characterize all the protoplasm here observed.

Streaming protoplasm is of a very liquid consistency. The same is true of young, actively growing plasma such as that found in the developing oögonium and the embryo of *Fucus*. The increase in consistency of the *Echinarachnius* ovum following the entrance of the sperm is not in harmony with this fact, nor is it what one would expect; for the liquid condition of protoplasm enables the elaborate chemical reactions of an active cell to take place. In the ripe egg, awaiting fertilization, metabolism is reduced to a minimum, so that a liquid state is not needed as it is for the complex chemical activities and interchange of substances which go on in the developing egg and growing embryo. In accordance with this is the fact that normal passive protoplasm in mature eggs of *Fucus* and *Echinarachnius* ova is quite viscous. (In ripe and resting seeds the protoplasm becomes almost solid.)

There is no reason for believing that any living protoplasm is naked. The surface membrane of protoplasm is at all times a definite morphological structure. The capacity to form such a pellicle is one of the characteristic properties of the living substance and is retained to a very late stage in dying protoplasm.

The membrane formed in repairing a tear is of the same character as the original. This is not true in those instances where the wall is of cellulose, as in pollen-tubes and plant embryos; for the enclosing surface layer of protoplasm is but a transformed portion of the living substance, the result of an immediate conversion of liquid plasma into a rigid gel of greater molar concentration. (Pfeffer, 1891, p. 194). It is worthy of note that protoplasm possesses not only the capacity to form a membrane but that kind of a membrane characteristic of a particular organism or of a particular stage of development. This suggests that the process is to some degree controlled from within.

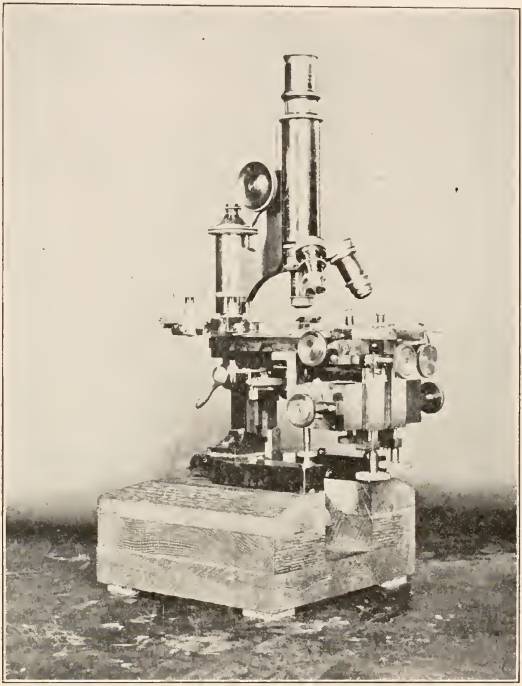
That contact with a *certain* medium is not necessary for membrane formation is evidenced from the fact that streaming myxomycetes form membranes whether dissected on a dry coverslip or in a hanging drop, and that the pellicle of the *Fucus* ovum is repaired whether it is torn open in the jelly-mass of the oögonium or in sea-water. Thus does it appear that contact with some medium is not a prerequisite to membrane formation, the lack of a membrane being the stimulus. Membranes are formed on any free protoplasmic surface. The general belief is that this is a purely physical process, and owes its origin to surface forces. It is somewhat disturbing to this strictly mechanistic conception that the capacity for membrane formation ends with death. (Pfeffer (1877) has shown experimentally that a membrane may be formed after death. Chemistry could give us many such instances. In neither case is the membrane a natural one.) Surface forces certainly come into play, but the capacity for membrane formation, and beyond doubt the factor determining the kind of membrane formed, is dependent upon the physiological state of the protoplasm, which, in a sense, means that the ultimate control lies within the cell; though, of course, not beyond the purely physical and chemical properties of the living substance.

The prevailing idea of a cell wall is that it is a dead structure and, therefore, incapable of change or response to environment. This is probably true of the cellulose walls of older cells, but not of the distensible, glutinous membranes and egg-walls here discussed nor of the cell-walls of meristematic tissues in general. The suggestion that the wall of a degenerating ovum may and does undergo, with the egg-protoplasm, a change which permits either its going into solution or its ready rupture by internal osmotic pressure, is not without experimental support. Protoplasmic membranes are an intimate part of the living substance and susceptible to the same changes in environment. (Bayliss, 1916, p. 115.)

Normal protoplasm, in all cases studied by the writer, does not mix with water. This declaration is contrary to that of Chambers (1917*a*, p. 2). If the decision is to rest on evidence gained solely from microdissection then miscibility of the protoplasm is a consequence of degeneration. Chambers's statement, however, rests not only on microdissection but on the introduction of water into the cell by the mercury injection method as well. Miscibility precludes the presence of a membrane. Normal protoplasm is always capable of membrane formation. Therefore, normal protoplasm can at no time be miscible. The dissection of a great many eggs supports this conclusion.

To the highest powers of the microscope, with ordinary illumination, protoplasm is a homogeneous, structureless solution. Its colloidal nature becomes evident on using the dark ground illuminator. Protoplasm is probably an emulsoid hydrosol, *i. e.*, a colloid in which both phases are liquid, one of them—the dispersion medium—being water.

Any definite statement on the ultimate structure of protoplasm must be expressed in physicochemical terms, and based upon observations made with the ultra-microscope. The behavior of protoplasm under dissection, however, throws considerable light on the gross structure of the cell contents. In distinguishing between gross and ultimate structure the writer has in mind, under the former, a haphazard arrangement of the minute subdivisions or centers of activity, of the living substance which are at any one moment in differing physiological states; while an ultimate structure connotes a definite arrangement of molecules or of the infinitesimal particles seen through the ultramicroscope—the microns or inferred amicrons of which all colloidal systems are composed.



Description of Photograph.

A two-needle Barber pipette holder attached to a Leitz microscope.

The most remarkable feature of life within a cell is the coexistence of a great number of reactions, constantly going on without one interfering with the other. This demands the division of the contents into innumerable chambers or centers of activity. Thus does the protoplasm possess, in a sense, structure. The presence of these miniature laboratories is well illustrated by the behavior of disintegrating ova in which the dissemination of the inclusions is at times in certain regions extremely rapid, and in others non-existent (Figs. 1 and 2). The difference in the physiological state of the protoplasm at any one moment is responsible for the variety of behavior of eggs from the same oögonium and of parts of the same ovum. This difference is to be expected if protoplasmic activity takes place in pulsations, which are neither synchronous among the eight eggs of one oögonium nor rythmic in any single ovum. The cell is, then, a laboratory in which many different chemical reactions are constantly going on, kept free from one another by boundaries of some kind. (Hofmeister, 1901.)

Structure in protoplasm is secondary to activity. Upon the chemical nature of the substances does the life of the cell depend rather than on their arrangement. What physical structures may exist are of a transitory nature. This interpretation does not preclude the all-important organization upon which the continuance of these activities depends.

SUMMARY.

1. Protoplasm is an emulsion colloid normally in the sol state.

2. The density of protoplasm varies from the very liquid state of young *Fucus* oögonia and embryos and of streaming protoplasm in myxomycetes and pollen tubes, to the quite viscous condition found in mature and resting eggs of marine organisms.

3. There is a rapid increase in viscosity of the *Fucus* egg during the last stages of its ripening, which is, on fertilization, followed by a return to the liquid consistency characteristic of active, growing proptolasm.

4. The plasma membrane is a definite morphological structure, constantly and repeatedly capable of repair through the conversion of the fluid protoplasm into a hyaline layer of greater molar concentration. This film of gel is exceedingly elastic, pliable and glutinous.

5. The surface layer, like the interior cytoplasm, seems to be capable of alteration with changes in environment.

6. The capacity for membrane-formation is one of the last essential properties of the living substance to be lost. It is lost only at death.

7. The kind of membrane formed is apparently identical with the parent membrane (except in the case of escaped protoplasm from cells which possess a cellulose wall).

8. The formation of a membrane is probably a purely physical process, but is dependent upon the physiological condition of the protoplasm. It is not dependent upon the surrounding medium.

9. The amount of physical disturbance that protoplasm can be subjected to before showing signs of injury varies from that of the immature *Fucus* ovum, where it is exceedingly great, to that of the ripe egg where it is very slight, often no more than a touch sufficing to cause disorganization.

10. Gelation of the plasma always takes place in time and is hurried by dissection. It is accompanied by degeneration.

11. Normal protoplasm is at all times non-miscible in water. Miscibility of the plasma is an unfailing criterion of degeneration.

12. Dissolution of the *Fucus* ovum is the result of the disorganized cytoplasmic matrix going into solution with the surrounding water. This mixing may take place with the rapidity of an explosion, or slowly, and then either continuously or spasmodically.

13. The disintegration of the egg plasm is frequently localized, in that certain regions of the contents continue dissemination of the granules from the beginning, while others join in later, and still others never take part. This indicates a gross structure of the egg plasm, *i. e.*, the protoplasm is composed of many centers of activity in which different chemical reactions take place separated by protective partitions.

14. That any definite and permanent arrangement of the colloidal particles exists seems unlikely. Whatever structure, gross or ultimate, protoplasm may possess is secondary to chemical activity upon which the life of the organism depends.

The writer wishes to thank Professor Duncan S. Johnson for suggesting this study and for assistance during its progress.

To Dr. Robert Chambers the writer is indebted for helpful suggestions, and to Dr. E. V. Cowdry for the use of his Barber instrument.

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FURTHER STUDIES ON THE PRODUCTION OF FUNCTIONAL AND RUDIMENTARY SPERMATOZOA IN ROTIFERS.[1]

D. D. WHITNEY.

In a recent paper some general observations were recorded in regard to the dimorphic spermatozoa found in the parthenogenetically developed males of nine species of rotifers. Some of the stages in the development of the spermatozoa from the cells of the last spermatocyte-divisions in *Brachionus mulleri* were described and figured. No studies, however, were made on the nuclear material of the spermatozoa either to determine whether it was present in both kinds of spermatozoa or to determine in what part of the spermatozoa it was located.

Additional studies now have been completed in an attempt to observe these points. These studies have been made in considerable detail on the marine species, *Brachionus mulleri*, and in lesser detail on *Hydatina senta*, *Brachionus amphiceros* and *Diaschiza sterea*. As the earlier paper had some of the details of the formation of the spermatozoa of *Brachionus mulleri* and as this rotifer is one of the most convenient forms to maintain in the laboratory and also a very convenient one from which to obtain immature males the majority of the observations were made on material from this species.

Several killing and fixing fluids and stains were tried but Zenker's fluid followed by Delafield's hematoxylin proved to be the most satisfactory method for making clear mounts of the entire cells. The living males were crushed under a cover glass in culture water and favorable cells were selected under the microscope. The killing fluid was allowed to run under the cover glass and to remain from 10-20 minutes. Later the killing fluid was drawn out by filter paper and distilled water allowed to take its place. After the distilled water had been changed several times from under the cover glass Delafield's hematoxylin

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[1] Studies from the Zoölogical Laboratory, The University of Nebraska, No. 119.

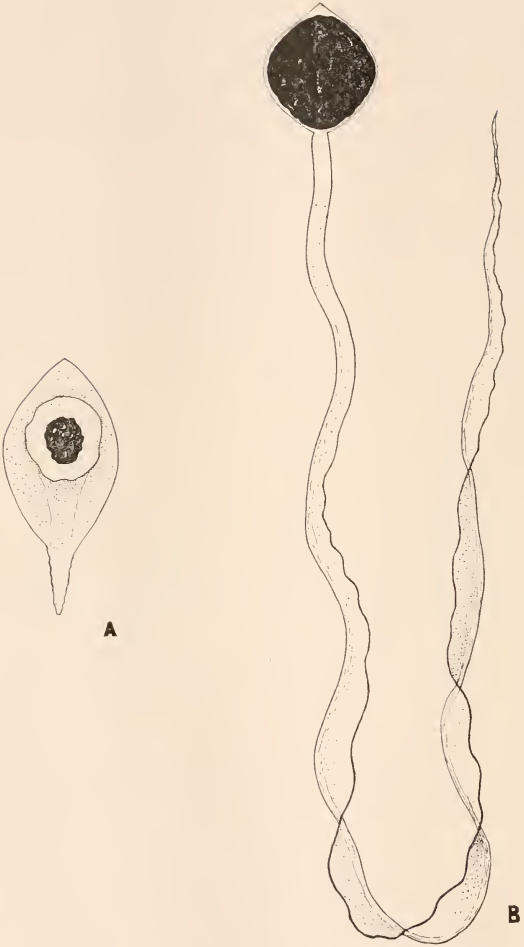


Fig. 1. *Brachionus mulleri.* *A*, normal spermatid showing the nucleus, chromatin, and the small tail; *B*, mature and normal spermatozoön showing the large chromatin mass in the head and the long vibratile tail with the undulating membrane. (Drawn by measurements from living and stained specimens with 1/12 oil immersion objective and a number 2 ocular.)

(diluted 1:1) was allowed to run under and to stain the cells for about two minutes. This was then washed out with tap water. All of these processes were carried on under the microscope in order to make certain that no mistakes of indentification of these cells should be made after the slides were finished. Although this is a tedious method it has the advantage of being perfectly reliable and furnishing accurate data concerning which there can be no doubts. Many other methods were tried but none were found to be as practical and as certain as this one.

The nucleus was first indentified in an early stage of the normal spermatid (Fig. 1, *A*) which later would have developed into the large motile spermatozoon (Fig. 1, *B*).

The degenerate sperm cells from the same male individual were examined next and each was found to contain a nucleus with chromatin material in it. This stage is shown in Fig. 2, *A*. This fact is of considerable interest because it demonstrates that in the division that forms the secondary spermatocytes there is evidently a division of the chromatin material and consequently all the secondary spermatocytes contain some of this chromatin material. One half of these secondary spermatocytes divide and form the normal spermatids but the remaining half of the secondary spermatocytes do not divide again but develop directly into the degernerate spermatozoa. The early stage of these degenerate spermatocyte cells are smaller in size than the normal cells of the same age but the nucleus is only slightly smaller than the nucleus contained in the normal spermatid cell as can be seen by comparing Figs. 2, *A* and 1, *A*.

The later development of each of these two kinds of cells is very different. In the normal cell the nucleus grows larger and larger until in its final stage it is several times its former size. From one end of the cell an outgrowth appears which grows longer and longer and finally becomes vibratile. This is the motile tail of the sperm cell. It is very long and large and has an undulating membrane along the greater portion of the dorsal side. Fig. 1, *B*, shows the entire matured spermatozoön containing the large mass of chromatin material in the head.

The development of the smaller and degenerate spermatocyte cells into the complete rudimentary spermatozoa is strikingly different from the development of the normal spermatid cells. After the early stage is passed the chromatin material increases in bulk very slightly and becomes composed of coarse granules

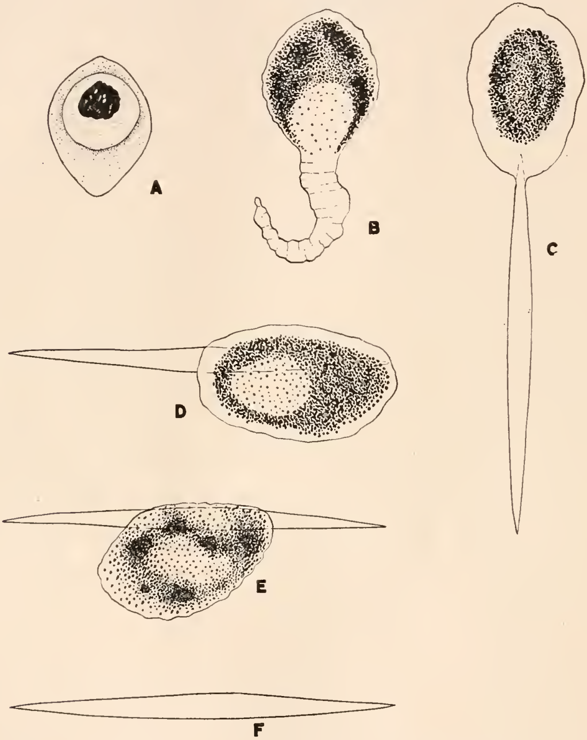


FIG. 2. *Brachionus mulleri.* *A*, early stage in the development of the rudimentary spermatozoön showing the chromatin in the nucleus. This cell is from the same male as the normal spermatid of Fig. 1, *A*, and is of the same age. *B*, a later stage showing the tail wrinkled and distorted by the fixing fluid; *C*, last stage showing the stiff and rigid tail attached to the head which contains the chromatin; *D*, the stiff tail forced through the chromatin to the opposite side of the head by pressure under the cover glass; *E*, the stiff tail forced through the opposite wall of the head by pressure under the cover glass; *F*, the stiff tail detached from the head. (Drawn to same scale as Fig. 1.)

loosely arranged and persists in this form throughout the remainder of the development of the cell. From one end of the cell a process extends which is somewhat flexible in its earlier development. Fig. 2, *B*, shows such an early stage in which the tail-like process has become curved and wrinkled after treatment with Zenker's fluid. Later this tail-like process becomes very rigid and stiff. So stiff that when the immature males are crushed this process by chance may be driven through the nuclear material to the opposite side of the cell as shown in Fig. 2, *C*, or in some instances it was even driven through the opposite wall of the cell as shown in Fig. 2, *D*. Normally this stiff process seems to be attached to one end of the cell. Later, however, when it is fully formed it becomes detached from the cell. This separation of the two parts of the cell occurs in the testis either before the male hatches from the egg or very soon thereafter. Fig. 2, *C*, shows the two parts just prior to separation.

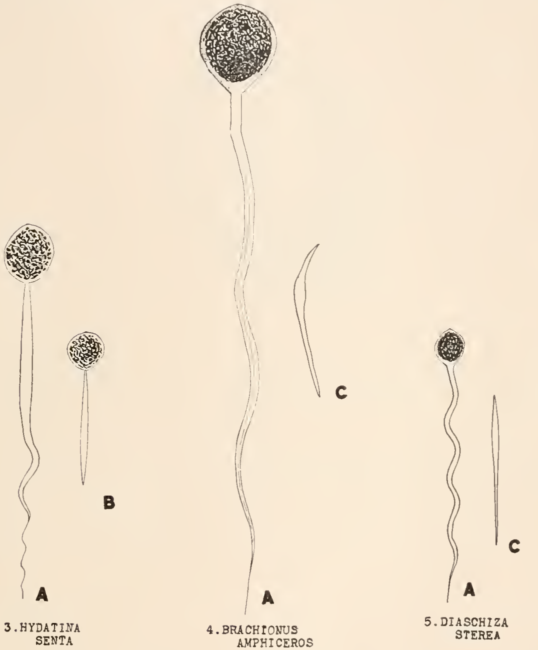
This stiff spindle-shaped part contains no chromatin material and probably is to be considered the rudimentary tail of the cell. It is immotile and much smaller than the tail of the motile spermatozoön. As it separates from the nucleated portion of the cell and is immotile one could naturally conclude that it is to be regarded as simply a degenerate or rudimentary tail which has no function. Whether the nucleated portion of the cell has any function is unknown but as it is also immotile it is also very probably functionless.

In the earlier studies the detached tails of the normal spermatozoa were erroneously considered to be the entire spermatozoa. When the males were crushed under a cover glass these tails were extruded and were able to move about in the water for a considerable length of time. When recently, however, these so-called spermatozoa were stained no nucleus or chromatin material could be found in any of them. As they are the functional spermatozoa it was realized that there must be a nucleus and chromatin material somewhere in them.

It was recalled that in previous observations some males had been seen to extrude a few of these motile bodies which had a large swelling on the anterior end. At that time these motile bodies were considered to be the immature spermatozoa. When more careful observations were made both upon such living cells and upon stained ones it was readily seen that these cells were not immature stages but were the mature stages of the spermatozoa. The heads are quite large in proportion to the diameter of the tail, Fig. 1, *B*, and are easily separated from the tails. In the testis of the male these spermatozoa are in clusters and in each cluster each spermatozoön is attached to some sort of a central tissue in the cluster. Normally in copulation with the young females a few of these spermatozoa become detached from the central tissue and are extruded into the body cavity of the female. When, however, the males are crushed under a cover glass nearly every head remains attached to this central tissue but the tails break off from the heads and are immediately extruded in a writhing mass. If the males are sufficiently crushed the mass of spermatozoa heads will also be extruded together with other cells of the broken-down tissues. If very young males are taken from the eggs and are crushed all of the immature sperm cells with the tails attached will be extruded. The ripe spermatozoa resemble very closely these immature spermatozoa except their heads are more rounded and the tails are somewhat longer.

The males of another rotifer, *Hydatina senta*, were crushed and the spermatozoa reëxamined. A few males shed both kinds of spermatozoa with the tails attached to the heads. These were stained and the heads of each kind were found to contain chromatin material similar to the heads of the spermatozoa of *Brachionus mulleri*. These are shown in Fig. 3. Twenty-five males were crushed and out of the many hundred spermatozoa ejected only about 6–8 entire spermatozoa of each kind with the tail attached to the head were found. *Hydatina senta* males are the most favorable material thus far examined in which to see the two kinds of complete spermatozoa in a mature male. In *Brachionus mulleri* these two kinds of complete spermatozoa could only be found in the unhatched and immature males.

The spermatozoa were examined from mature males of two additional species of rotifers, *Brachionus amphiceros*, and *Diaschiza sterea*. The entire normal spermatozoa with the tails attached to the heads were found in each species. Upon staining the heads showed their chromatin contents. Only the tails of the rudimentary spermatozoa were found and they showed no chromatin material in them when stained. Sketches of these spermatozoa and tails are shown in Figs. 4 and 5.



FIGS. 3–5. Dimorphic spermatozoa and tails of three other species of rotifers. *A*, larger normal and motile spermatozoa showing the head and the contained chromatin; *B*, smaller, rudimentary and non-motile spermatozoön showing the chromatin in the head; *C*, detached tails of rudimentary spermatozoa. (Drawn with 4 mm. objective and number 2 ocular.)

The spermatozoa of the other species of rotifers enumerated in the former paper have not been reëxamined because these forms are not available at this season of the year.

As the stained slides of the sectioned unlayed fertilized eggs of *Hydatina senta* were available for study they were examined to determine the size and condition of the head of the spermatozoön after it had entered the egg. The egg examined was sectioned while inside the body of the female and although it had the thick secretion around it which later would have shrunken to form the heavy external covering or shell it was readily sectioned at this early stage. The sperm head is seen near the nucleus of the egg (Fig. 6). It is about the size of the head of the normal spermatozoön as can be seen by comparing it with Fig. 3, but contains denser chromatin material and is perfectly round in form.

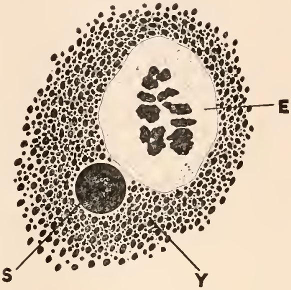


FIG. 6. Portion of a fertilized egg of *Hydatina senta*. *E*, egg nucleus containing chromosomes; *S*, sperm-head; *Y*, yolk granules of the egg. (Sperm-head and the outline of egg nucleus drawn to same scale as in Figs. 3–5. Yolk granules and chromosomes drawn freehand without measurements.)

Dimorphic spermatozoa have been studied in considerable detail in some of the Mollusca and in some of the Lepidoptera by several workers. Among these Goldschmidt and Gatenby recently have come to the conclusion that the atypical or abnormal spermatozoa are wholly functionless and the latter is of the opinion that such spermatozoa have no significance in regard to sex regulation. According to their conclusion any spermatozoa and in some cases all spermatozoa of these forms studied may become degenerate if the male individual is in a certain physiological condition.

In the phylloxerans and in the rotifers the case is quite different. In each and every male a certain percentage of the sperm cells degenerate and a certain percentage of the sperm cells develop normally, thus giving a definite ratio of degenerate and normal sperm cells. In the phylloxerans this ratio has not been determined but in the rotifers there is one degenerate sperm cell to two normal sperm cells. As all the fertilized eggs in both phylloxerans and rotifers develop into female young it seems safe to conclude, as Morgan has already concluded, that the degenerate sperm cells are the male-determining ones and that the normal sperm cells are the female-determining ones.

SUMMARY.

1. The normal and motile spermatozoa of *Brachionus mulleri*, *Brachionus amphiceros*, *Hydatina senta* and *Diaschiza sterea* possess large heads in which is located the chromatin material.

2. The motile bodies of seven other species of rotifers which were figured and considered as the normal spermatozoa in the former paper were probably only the tails of such normal spermatozoa.

3. The degenerate and immotile spermatozoa as seen in *Brachionus mulleri* and *Hydatina senta* also possess heads in which there is chromatin material.

4. The stiff processes, called rudimentary spermatozoa in the former paper, are now considered to be not the complete rudimentary spermatozoa but only the degenerate tails of these rudimentary spermatozoa.

5. The sperm head in the fertilized egg of *Hydatina senta* is about the same size as the head of the normal spermatozoön of this species.

6. The four species of rotifers enumerated above together with the seven other species described in the former paper constitute eleven species of rotifers in which both normal and rudimentary spermatozoa have been found to occur.

Zoölogical Laboratories,
The University of Nebraska,
Lincoln, Nebraska, January 5, 1918.

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BIOLOGICAL BULLETIN

EFFECT OF ENVIRONMENT UPON INHERITED CHARACTERS OF *HYDATINA SENTA*.

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Introduction.

Several years ago it was discovered (Shull, 1915) that two distinct parthenogenetic lines of the rotifer *Hydatina senta*, one from England, the other from Nebraska, differed in certain physiological (and perhaps structural) characters: (1) the English line laid smaller eggs than the Nebraska line; (2) the English line habitually laid a large percentage of its eggs attached to the surface film of the water, while the Nebraska line laid most of its eggs at the bottom or sides of the vessel; (3) the eggs of the English line required longer to develop than did those of the Nebraska line; (4) and when the rotifers were killed in Bouin's fluid the foot was seldom, or only slightly, retracted in the English line, but considerably retracted in the Nebraska line.

When crosses were effected between these two lines, the F_1 lines and F_2 lines were all indistinguishable from the English line in all the above-named characteristics. It seemed as if segregation and recombination had failed, and that in some way the four characters were rigidly associated one with another.

At first it was regarded as possible that the four characters were not really distinct, but were different manifestations of a single (physiological) character. That character might have been a greater permeability of the cells in one line than in the other. Thus, if the Nebraska line were more permeable to oxygen, the increased metabolism might make its eggs larger. For the same reason the eggs of the Nebraska line might develop in less time. In like manner it might be that the Nebraska rotifers, able to get the required amount of oxygen at the bottom of the dish where the oxygen in solution was less abundant, lived most of the time at the bottom and laid their eggs there; whereas the less permeable English rotifers were forced to swim to the surface where dissolved oxygen was presumably more abundant, and laid eggs at the surface film. And if the foot muscles of the Nebraska line were more permeable to the killing fluid than were those of the English line, the greater contraction of the muscles of the former might thereby be explained.

It was possible to test the correctness of the above assumptions, in part, by artifically altering the expression of the inherited characters through changes in the environment. A number of experiments were performed to this end. However, before they were completed, an F_3 generation was obtained in which the association of the four inherited characters was broken. In this and the succeeding generations each one of the four characters was separated at least once from the others with which it was associated in the original lines.

Thus was proven that the four characters were not merely different expressions of one character. The experiments designed to test their separateness or singleness were, therefore, not completed, and were not published. It has now become necessary, however, to refer to certain of the results, and they are here described in the incomplete form in which they were left. Along with them are several experiments on the viability of fertilized eggs, as affected by external conditions. These are of interest to the experimenter from a practical standpoint, and also in relation to popular ideas concerning the fertilized eggs of *Cladocera.*

Experiments.

Effect of oxygen upon the laying of eggs at surface and bottom of water.

On each of the dates named in Table I., approximately equal numbers of females of *Hydatina* were placed in two dishes. In one was placed water oxygenated by vigorously shaking it in an atmosphere having a high percentage of oxygen. The dish was then set, uncovered, under a bell jar in which was confined an atmosphere containing the same high percentage of oxygen as that with which the water was first saturated. Since the bell jar was being used daily for other experiments, the percentage of oxygen used was not always the same. From May 27 to June 22, and on July 8 (see Table I.), the atmosphere contained 40 per cent. of oxygen; on all other dates 60 per cent.

In the other of the two dishes was placed untreated water. This dish was also set under a jar sealed at the edges, to prevent excess of evaporation, but in ordinary air.

After 20 to 40 hours the dishes were removed and the number of eggs at the surface film and at the bottom counted. As shown in Table I., the eggs are much less abundant at the surface film in the presence of much oxygen than in air.

TABLE I.

Showing the Number of Eggs Laid at the Surface Film and at the Bottom of the Dish by Rotifers Placed in Air and in an Atmosphere Containing an Excess of Oxygen.

Date.	Air.		Excess of Oxygen.	
	Number of Eggs at Surface.	Number of Eggs at Bottom.	Number of Eggs at Surface.	Number of Eggs at Bottom.
May 27	12	5	1	15
29	37	48	16	55
June 3	22	22	16	27
22	2	42	0	25
28	62	20	19	49
29	8	25	7	29
30	43	85	18	121
July 1	38	19	17	27
2	40	33	34	39
3	45	6	14	41
4	42	36	23	76
6	12	5	8	22
8	23	10	12	22
Total	386	356	185	548
Percentage at surface	52.0		25.2	

Effect of oxygen upon the size of parthenogenetic eggs.

Eggs laid by rotifers in oxygenated water, and in untreated water, were obtained in the following manner. Three or four young females, due to begin egg laying in 6 to 12 hours, were placed in each of two watch glasses. One lot was immersed in water saturated with an atmosphere of which 40 per cent. or 60 per cent. was oxygen, and then set under a bell jar in a similar atmosphere. The other lot was placed in spring water, and the dish was set under a closed bell jar in air. After 24 hours the watch glasses were removed, and all the eggs that were in a horizontal position were measured by means of a screw micrometer eye-piece. The measurements are given in units of the scale. They are directly comparable with the measurement of eggs in my former paper (Shull, 1915) since all the measurements were made with the same microscope and with the same lenses.

The mean length, mean breadth, standard deviation, etc., of the two lots of eggs are given in Table II.

TABLE II.

A Comparison of the Eggs Laid by the Rotifer Hydatina Senta in Oxygenated Water and Untreated Water.

	In Oxygenated Water.	In Untreated Water.	Difference.
Number of eggs measured......	100	100	0
Mean length of eggs...........	16.208 ± 0.028	16.098 ± 0.027	0.110 ± 0.039
Mean breadth of eggs.........	14.054 ± 0.021	14.021 ± 0.018	0.033 ± 0.028
Standard deviation of length...	0.409 ± 0.019	0.404 ± 0.019	0.005 ± 0.026
Standard deviation of breadth..	0.308 ± 0.015	0.266 ± 0.013	0.042 ± 0.019

The eggs in the oxygenated water were a trifle larger than those in the untreated water, though it can hardly be said that the difference is statistically significant. However, a difference that cannot be proven by statistical treatment to be significant is not necessarily insignificant. It seems to me not improbable that the difference in length is significant, but it is very small in comparison with the difference between English and Nebraska eggs described in my earlier publication (*op. cit.*).

Effect of oxygen upon the time of development of parthenogenetic eggs.

A number of egg-laying females were put into each of two dishes in the evening. In one dish was placed ordinary water, in the other water that had been oxygenated in the manner described in the preceding experiments. The females were removed after about an hour, but the eggs which they had laid were left in the dishes. The oxygenated water in one dish was then removed and replaced with fresh oxygenated water, and the dish was set under a bell jar in an atmosphere containing an excess of oxygen (40 or 60 per cent.). To insure that mechanical disturbance or accumulation of metabolic products did not affect the time of development of the eggs unequally, the water in the control dish was also drawn off after the rotifers were removed and replaced with fresh water. This dish was then set under a bell jar in air.

The next morning the two dishes were examined at intervals of 20 to 30 minutes, and the young rotifers removed and counted as they hatched. In this way the approximate time of development of the eggs was determined. In Table III. these times are collected in groups, to the nearest half hour. When these figures are treated statistically, they compare with one another as in Table IV.

Table III.

Showing the Time Required for Development of Eggs in Oxygenated Water and in Untreated Water.

	Number of Eggs Hatching in (Hours).						
	11.5.	12 0.	12.5.	13.0.	13.5.	14.0.	14.5.
Oxygenated water...........	7	10	15	23	9	1	
Untreated water............	7	8	12	15	14	4	1

Table IV.

Showing the Time of Development of Eggs in Oxygenated Water and in Untreated Water. Statistical Treatment of Data in Table III.

	In Oxygenated Water.	In Untreated Water.	Difference.
Mean time of development in hours......................	12.654 ± 0.104	12.803 ± 0.129	0.149 ± 0.165
Standard deviation of time of development...............	1.239 ± 0.074	1.497 ± 0.091	0.258 ± 0.117

The eggs in oxygenated water hatched in a trifle shorter time, and somewhat more uniformly, though the difference in each case is so small that it may be insignificant. The greater uniformity of the time of development in oxygenated water (second line of Table IV.) is not improbably significant.

Effect of Oxygen upon the Contraction of the Foot Muscles.

Rotifers were placed in water saturated with an atmosphere containing 40 per cent. of oxygen, under a bell jar containing a similar atmosphere, and kept there 24 hours. They were then removed and promptly killed in Bouin's fluid. Other rotifers from the same families were kept in ordinary water, under a bell jar in air, for 24 hours, then killed in Bouin's fluid.

The contraction of the foot muscles was noted in accordance with the following arbitrarily chosen degrees of contraction: (0) foot fully extended; (1) foot slightly contracted, toes bent to one side, but still visible; (2) foot considerably contracted, toes wholly concealed, but contraction limited to small region near toes; and (3) foot greatly contracted, region of contraction much greater than in preceding class. It is to be noted that this classification is not the same as that proposed for the English and Nebraska rotifers in my former paper (Shull, 1915). The descriptions there given were not applicable to the rotifers used in these experiments.

The degrees of contraction of the foot muscles of the two lots of rotifers is tabulated in Table V., and the statistical comparison of the figures in Table VI.

TABLE V.

Showing the Degree of Contraction of the Foot Muscles of Rotifers Kept in Oxygenated Water, and in Untreated Water, and Then Killed in Bouin's Fluid.

Degree of Foot Contraction.	Number of Rotifers of Given Foot Contraction.	
	In Oxygenated Water.	In Untreated Water.
0	35	21
1	97	111
2	64	64
3	2	2

TABLE VI.

Statistical Comparison of Data in Table V.

	In Oxygenated Water.	In Untreated Water.	Difference.
Mean foot contraction	1.16 ± 0.034	1.27 ± 0.030	0.11 ± 0.046
Standard deviation of foot contraction	0.64 ± 0.021	0.71 ± 0.024	0.07 ± 0.031

The statistical treatment is based on the assumption that each degree of contraction of the foot muscles differs from the degrees next to it by unity.

As shown in Table VI. there is less contraction of the foot muscles in oxygenated water than in untreated water, but the difference is rather small compared with its probable error and in view of the crudity of the method of measurement may be without significance.

Effect of Temperature upon the Laying of Eggs at Surface and Bottom.

Equal numbers of rotifers were placed in a number of dishes of water. Some of the dishes were kept at room temperature which was fairly constant at 20° to 22° C. Other dishes were kept much cooler by setting them on a window sill outside. A thermometer was kept beside the latter dishes, and was frequently read. In Table VII., which gives the number of eggs laid at the surface film and at the bottom, the stated temperature

Table VII.

The Number of Eggs Laid at the Surface Film and at the Bottom at Room Temperature and at Considerably Lower Temperatures.

Date.	Room Temperature.		Low Temperature.		
	Number of Eggs at Surface.	Number of Eggs at Bottom.	Number of Eggs at Surface.	Number of Eggs at Bottom.	Temperature in Degrees C.
Nov. 28.....	44	74	1	6	8
Nov. 29.....	20	59	0	14	7
			0	8	
			0	9	
			0	9	
Dec. 3.....	8	20	0	6	4
			0	10	
			0	4	
			0	2	
			0	0	
Dec. 4.....	15	22	0	0	2
			0	1	
			0	1	
			0	0	
Dec. 7.....	74	20	0	3	3
			0	1	
			0	1	
Dec. 8.....	6	20	3	7	12
			2	19	
			0	0	4
			0	3	
Totals......	167	215	6	104	
Percentage at surface....	43.7		5.5		

of these cool dishes is an estimated average. Because fewer eggs were to be expected at such low temperatures, several dishes were kept at low temperature for every one at room temperature.

There is no question here that the eggs are laid more largely at the surface when the temperature is high. The experiment was repeated on a small scale with the same result.

Effect of Temperature upon the Viability of the Fertilized Eggs.

Freezing.—Freezing of fertilized eggs was designed as a practical measure only, in order to induce those eggs to hatch which would not otherwise hatch. The attempt failed, however, as shown by the following tests.

In the first experiment about 244 eggs that remained unhatched after hatching had ceased for 12 days in the lot of eggs to which they belonged, were divided into two approximately equal groups. One group was frozen over night by immersing, in a closed vessel, in a brine-ice mixture and the other was kept in water at ordinary temperature. Of the frozen lot none hatched in 19 days thereafter. Of the control lot at room temperature, one hatched in 10 days after the beginning of the experiment, none thereafter Freezing did not facilitate the hatching.

In the second experiment about 230 eggs that remained unhatched for 14 days after hatching of the eggs in the same lot had practically ceased were similarly divided into two groups, one of which was frozen over night and the other kept at room temperature. None of these eggs in either lot hatched in 19 days, after which time observation ceased.

From these experiments it appears that fertilized eggs not ordinarily capable of hatching can not be made to hatch by freezing.

Low Temperature Above Freezing.—Two lots of fertilized eggs from the same source and of approximately equal numbers were kept at different temperatures from the time they were laid until hatching was nearly complete. The eggs were laid between November 29 and December 8. One lot was kept at room temperature. The other was set outside on a window sill where daytime temperature, as shown by a thermometer placed beside the dishes, ranged from 2° to 12° C. The air temperature was much below this, but was moderated in the location of the eggs by a steam radiator near the window sill inside. It appears that the water in which the eggs were placed never froze and hatching occurred during this period of low temperature. From December 22 to December 25 the atmospheric temperature was considerably above freezing but was much colder thereafter. On January 3 the "cold" dish was removed to room temperature, but hatching had been nearly completed before that time. Table VIII. shows the number of eggs that hatched.

TABLE VIII.

Showing the Effect of Room Temperature and Lower Temperatures Upon the Hatching of the Fertilized Eggs of Hydatina Senta.

Date of Beginning Experiment.	Warm.			Cold.		
	Number of Eggs.	Date of Hatching.	Number Hatched.	Number of Eggs.	Date of Hatching.	Number Hatched.
Nov. 29....	44	Dec. 6	1	43	Dec. 23	1
Dec. 2....	10	Dec. 7	1	10	Dec. 23	2
					Dec. 24	2
					Jan. 5	1
Dec. 8....	43	Dec. 10	2	44	Dec. 10	1
					Dec. 12	2
					Dec. 24	1
Total number hatching..........			4			10

Low temperature appears to favor the hatching of the eggs, not as an after effect, but during the period of low temperature.

Effect of Oxygen upon the Viability of the Fertilized Eggs.

Each of four lots of eggs from a single source was divided into two equal parts. One was placed in water of high oxygen content (saturated with an atmosphere of which either 40 per cent. or 60 per cent. was oxygen) and set under a bell jar enclosing an atmosphere containing the same proportion of oxygen as that with which the water was originally saturated. Atmospheres of 40 per cent. oxygen were used until July 7, 60 per cent. thereafter. This dish was removed from the bell jar daily and examined for hatching rotifers. The water was drawn off the eggs after examination and replaced with fresh oxygenated water and the dish returned to the bell jar.

The other dish was filled with untreated water which was drawn off daily and replaced with fresh water.

The hatching of the eggs is recorded in Table IX.

Table IX.

Showing the Effect of Oxygenation of the Water Upon the Hatching of the Fertilized Eggs of Hydatina Senta.

Experiment.	Date of Starting Experiment.	Oxygenated Water.			Untreated Water.		
		Number of Eggs.	Date.	Number Hatching.	Number of Eggs.	Date.	Number Hatching.
A.......	July 1, 1915	24	July 3	1	24	July 2	1
			7	1		7	1
			9	1		8	2
						12	1
				3			5
B.......	July 2, 1915	30	July 6	4	30	July 5	4
			10	1		8	1
			21	1		9	1
						10	1
						17	1
				6			8
C.......	July 4, 1915	79	July 5	1	78	July 8	1
			9	4		9	5
			10	5		10	4
			11	2		11	1
			12	3		12	3
						13	1
						14	1
				15			16
D.......	July 7, 1915	100	July 10	1	100	July 8	1
			11	2		9	1
			12	2		10	1
			12	5		11	3
			14	2		12	1
						13	8
						14	3
						21	1
						23	1
				12			20
Grand total....	....			36	....		49

The eggs in oxygenated water show a somewhat lower viability in every case.

Effect of Drying upon the Viability of the Fertilized Eggs.

Dried for a Short Period.—A lot of cross-fertilized eggs—eggs laid by females of line B fertilized by males of line A of my former experiments (Shull, 1913)—were kept for seven weeks to allow hatching to take place. Practically all the hatching occurred in the second to fourth weeks of this period, almost none in the last three weeks. The 446 eggs (about two thirds of the original lot) which remained unhatched after seven weeks were divided into two nearly equal groups. One half was allowed to become dry December 8 and remain dry about 13 hours, after which it was remoistened. The other half was kept wet. The subsequent hatching of eggs from these two groups is recorded in Table X.

TABLE X.

Showing the Effect of Drying Eggs for a Short Period Upon the Proportion that Hatch in a Cross-Fertilized Lot of Eggs of Hydatina Senta.

Eggs Kept Wet.		Eggs Dried and Remoistened.	
Date.	Number Hatching.	Date.	Number Hatching.
Dec. 9	0		
10	0	Dec. 10	0
11	1	11	0
12	0	12	0
13	0	13	2
14	0	14	3
15	0	15	1
16	0	16	8
17	0	17	4
18	1	18	5
19	0	19	4
20	0	20	7
Total.	2		34

Observations necessarily stopped December 20, but it seems likely that even more would have hatched among the eggs that were dried. Drying for a short period either favors hatching of eggs that would not otherwise have hatched or hastens the hatching of eggs whose hatching would otherwise have been spread over a long period.

The above experiment was repeated with a lot of eggs from the reciprocal cross of the foregoing—eggs laid by females of line A fertilized by males of line B. The original lot of eggs was kept seven weeks to allow of hatching. During the last three weeks of that time very little hatching occurred. The 238 eggs that remained (less than half of the original lot) were divided into two equal parts, one of which was allowed to become dry and remain so for 13 hours on December 4. The subsequent hatching is recorded in Table XI. The results confirm the conclusions drawn from Table X.

Table XI.

Showing the Effect of Drying Eggs for a Short Period Upon the Proportion that Hatch in a Cross-Fertilized Lot of Eggs of Hydatina Senta. The Cross was the Reciprocal of that in Table X.

Eggs Kept Wet.		Eggs Dried and Remoistened.	
Date.	Number Hatching.	Date.	Number Hatching.
Dec. 5–20	0	Dec. 7–15	0
		16	1
		17	1
		18	16
		19	2
		20	1
Total............	0		21

In another experiment of this kind inbred eggs (eggs laid by females fertilized by males of the same line) of a line in which a relatively small proportion of the eggs normally hatched were used. The eggs were kept five weeks, during the last 12 days of which time no eggs hatched. The eggs that remained unhatched were divided into two lots which, by mistake, were made unequal. One lot, consisting of about 130 eggs, was dried over night; the other of 106 eggs, was kept wet. The subsequent hatching of these eggs is recorded in Table XII.

Table XII.

Showing the Effect of Drying for a Short Period Upon the Hatching of Inbred Eggs of Hydatina Senta.

Eggs Kept Wet.		Eggs Dried and Remoistened.	
Date.	Number Hatching.	Date.	Number Hatching.
Feb. 25 to Mar. 8 ..	0	Feb. 24 to Mar. 4 .	0
Mar. 9...........	1	Mar. 5..........	1
Mar. 10 to 14......	0	Mar. 6 to 14......	0

Unlike the cross-fertilized eggs of Tables X. and XI., drying for a few hours neither increased the number of eggs that hatched nor hastened their time of hatching.

The foregoing experiment was repeated with inbred eggs of a line that regularly hatched more than half its eggs. The eggs remaining unhatched after five weeks were divided into two lots, one of which was dried over night and then remoistened. Though both were kept three weeks, not one egg in either lot hatched. Drying neither increased nor hastened the hatching.

Dried for Periods of Moderate Length.—These experiments differed from the foregoing in that all of the eggs were dried in one half of the experiment, instead of only those which failed to hatch under ordinary conditions. Inbred eggs were used, and drying occurred about the time when hatching was due to begin, that is, a week after the eggs were laid. Hatching began three or four days after the eggs were remoistened. Observations were continued for a month after remoistening. One lot of eggs was kept wet as a control, one was dried over night, one dried two weeks, and a fourth dried four weeks. The experiment was performed three times. The totals, without daily records, are given in Table XIII. The second division of this table really belongs to the preceding section of this paper, since it involves only a short period of drying, but it seems best to retain it here for the sake of comparison.

Table XIII.

Showing the Effect of Drying for Various Periods Upon the Viability of the Fertilized Eggs of Hydatina Senta. Inbred Eggs Were Used.

Experiment.	Eggs Kept Wet.		Dried Over Night.		Dried Two Weeks.		Dried Four Weeks.	
	Number of Eggs.	Number Hatching.	Number of Eggs.	Number Hatching.	Number of Eggs.	Number Hatching.	Number of Eggs.	Number Hatching.
A	75	40	78	35	76	18	72	0
B	55	26	41	6	50	12	53	0
C	39	23	33	19	38	6	27	0
Total.......	169	89	152	60	164	36	152	0
Percentage hatching ..	52.6		39.4		21.9		0.0	

There is plain indication in these results that drying, even for a short time, reduces the number of eggs that will hatch when again placed in water; and that the longer the period of desiccation, up to the limit of complete inhibition, the fewer the eggs that hatch.

Dried for a Long Period.—No experiment with control, involving a longer period of desiccation than four weeks, was performed; but that some eggs could withstand longer desiccation was shown. One lot of 344 cross-fertilized eggs and another of 100 inbred eggs, all from sources not used in the experiments described in this paper, were kept in dried condition from June 29 to March 23, or about nine months, when they were remoistened. In about three weeks thereafter three eggs of the first group and one of the second hatched.

All these eggs simply rested on the bottom of a watch glass when dried. It is not improbable that when the eggs, on drying, are supported by mud or sand they may remain desiccated longer and still hatch when remoistened. But even in mud the possible period of desiccation is not indefinitely long; for out of a lot of fertilized eggs in dried mud I have secured numerous young rotifers after three months of desiccation, but no eggs hatched from this lot after two years.

Summary and Discussion.

In former papers (Shull, 1913, 1915) the inheritance of size of parthenogenetic eggs, the time of development of parthenogenetic eggs, the place of laying parthenogenetic eggs (surface film or bottom), the viability of fertilized eggs (proportion that hatch), and the contractility of foot muscles was described. In this paper is shown to what extent these inherited characters may have been modified by such external agencies as temperature, oxygen, and desiccation.

It was found that if the water in which the rotifers live was exposed to an atmosphere containing more than the usual proportion of oxygen, a greater proportion of the eggs were laid at the bottom of the vessel. Under ordinary conditions the rotifers probably come to the surface because of the greater quantity of dissolved oxygen there. Those lines which normally lay their eggs mostly at the bottom probably either require less oxygen or get their oxygen more easily than lines which lay their eggs at the surface film. The hereditary character involved may therefore be the oxygen requirement or the permeability to oxygen.

If the rotifers were kept at a low temperature, their eggs were laid more largely at the bottom. This may be due to the greater concentration of oxygen in the water at low temperature, so that it is unnecessary to come to the surface so much; or to the low metabolism and hence low oxygen requirement of the animals; or to both of these reasons.

Excess of oxygen increased the size of the parthenogenetic eggs only very slightly, or not at all. Excess of oxygen decreased the time of egg development only very slightly, or not at all. It may have made the time of development a trifle more uniform, though this is hardly proven.

Rotifers kept in oxygenated water showed, when killed in Bouin's fluid, a trifle less contraction of the foot muscles than did other rotifers, though the difference was too small to constitute a proof of the action of oxygen.

Since the place of laying the eggs (surface or bottom) was so much more greatly affected by oxygen than were the other three characters tested, it would seem rather improbable even if the genetic results (Shull, 1915) had permitted such an assumption, that the several characters in which the English and Nebraska lines differ as described in the introduction to this paper, were really but a single character with several manifestations. The results described in this paper, therefore, harmonize with the genetic results previously obtained.

Freezing fertilized eggs that had remained unhatched for five to seven weeks did not induce any of them to hatch later. However, when eggs were kept from the time of laying at a low and variable temperature above freezing, more of them hatched than when kept at room temperature.

When fertilized eggs were kept in oxygenated water, a somewhat smaller proportion of them hatched than in untreated water.

Cross-fertilized eggs which had remained for seven weeks under ordinary conditions without hatching were dried over night and then remoistened. A considerable number of them were thereby caused to hatch. Inbred eggs, however, when tested in the same way, did not respond to drying. Even inbred eggs that were dried shortly after they were laid (not merely those which failed to hatch otherwise) did not show any increase in the proportion of viable eggs. Indeed, drying had just the opposite effect on inbred eggs. Even when the eggs were kept dry only a few hours, the percentage of them that hatched was reduced; and the longer the eggs were kept dried the fewer of them hatched. Those that remained dry for four weeks did not hatch at all.

Other lines were not as sensitive to drying, for out of one lot of eggs that were dry for nine months, several eggs hatched when remoistened.

In view of the results of desiccation of inbred eggs, it is conceivable that the hatching of cross-fertilized eggs after drying was due merely to the hastening of the development of eggs whose hatching would otherwise have been spread over a long period. If we had for comparison only the experiments with inbred eggs which were dried immediately after laying, and those with cross-fertilized eggs that were dried after they had been allowed abundant time in which to hatch and had not done so, the conclusion just stated would seem not merely conceivable, but probable. However, since inbred eggs were also dried after their normal hatching period was past, and failed to hatch subsequently, whereas cross-fertilized eggs thus treated did hatch, I am inclined to believe that drying for a few hours actually caused some cross-fertilized eggs to hatch which would not have done so without drying. From the physiological viewpoint, such a difference between inbred eggs and cross-fertilized eggs is not at all improbable.

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THE EFFECT OF SELECTION UPON THE SEX-RATIO IN DROSOPHILA AMPELOPHILA.

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It is a well-known fact that sex-ratios in most animals approximate equality. The ratio is seldom one of exact equality, however, and the preponderance may be in favor either of the males or the females. The variation from equality is fairly constant for the species. For man the ratio has been found to be 100 females to 105 males; for the horse, 100 to 98; for the cow, 100 to 107; for the sheep, 100 to 97; for the pig, 100 to 111; for the dove, 100 to 105; and for the hen, 100 to 94. These variations are so constant that they cannot be attributed to chance and they are irregularities for which our present theories of sex determination offer no adequate explanation.

The writer has attempted to determine the sex-ratio of the fruit fly, *Drosophila ampelophila*. The determination was made from three unrelated stocks in which the best possible environmental conditions were provided. All matings were in single pairs. Over 35,000 flies were examined and the ratio was found to be 100 females to 95 males.

Moenkhaus tested the effect of selection upon the sex-ratio of *Drosophila ampelophila* and decided that the sex-ratio in this species is "amenable to selection." If it be true that the relative number of males and females in a strain can be varied by selection, the present theories of sex determination must be somewhat modified. Although this fact would not necessarily disprove the theories of sex determination, it would necessitate the assumption of an hypothesis of selective fertilization or of differential mortality or viability of the determining elements.

In view of the vital bearing of Moenkhaus's findings upon the theories of sex determination, it has been deemed worth while to repeat his work.

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[1] Contribution No. 159.

Methods and Material.

The selection experiments were carried out upon three unrelated stocks. The stock for the first experiment was collected in Bloomington, Ind.; for the second, in Saratoga, Ind.; and for the third, in Warsaw, Ind. In the first two cases the experiments were started as soon as the stocks were collected from nature but the Warsaw stock was inbred in the laboratory for about six months before the experiment was started. At all times the greatest care was exercised to prevent contamination. The flies were provided with an abundance of food and care was taken to examine all the offspring of each pair. All matings were single pair matings.

Each experiment was conducted upon the offspring of a single pair of flies, that is, for the first experiment, a virgin female was selected from the Bloomington stock and mated to a brother. From among the offspring of this pair of flies, the parents of the two strains were obtained. The one strain in which there was selection for a relatively high number of females in comparison to the number of males, has been called the "high" strain and the one in which the selection was in the opposite direction, has been called the "low" strain. Before selection was started, a sufficient number of flies were examined to obtain a fair estimate of the sex-ratio of the stock under consideration. Moenkhaus's high and low strains were not offspring of a single pair of flies but were obtained by selecting from nature, pairs which had unusual ratios in the desired direction. Furthermore, he knew nothing of the original sex-ratio of his strains before selection began.

In each generation an attempt was made to examine the offspring of ten pairs in each strain. Unsuccessful matings, usually due to poor food conditions, sometimes prevented this. In each generation, the pair which gave the most extreme ratio in the desired direction was used as the parent of the succeeding generation. At times, technical difficulties prevented the most extreme pair being used and in these cases the next best was used. Pairs showing extreme ratios but producing a small number of offspring were not used. Since it was not possible to know which pair had the most desirable ratio till all of the offspring of all the pairs in a generation were examined, the technique of the experiment was made somewhat difficult. This might have been overcome by isolating some males and females from each mating and then, after the counts for that generation were completed, mating the offspring of the pair giving the most extreme ratio. This would have prolonged the experiment, so to avoid delay, at about the eighth or ninth day after the young had begun to emerge, fifteen pairs were mated from among the offspring of each mating which was being examined. In this way, by the time one generation was finished, the next was almost ready to begin to emerge. Since ten matings in each strain were examined, this necessitated the mating of about300 pairs of flies in each generation. A control was also carried in each experiment in which a few hundred flies were examined each generation.

PRELIMINARY EXPERIMENT.

This experiment was to test whether the age of the parents would in any way influence the relative number of males and females produced. Several matings were made and the adults were transferred to new bottles every eight days as long as they both lived. The age of the parents does not influence the sex-ratio as is shown by the results given in Table I.

TABLE I.

Showing Results of Experiment to Test the Effect of Age of Parent Upon the Sex-Ratio.

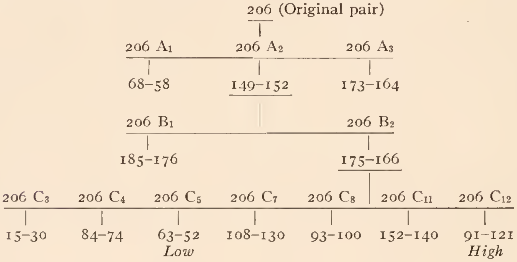
Culture Number.	Original Mating 1 Day Old.		First Transfer 11 Days Old.		Second Transfer 18 Days Old.		Third Transfer 26 Days Old.		Fourth Transfer 34 Days Old.		Fifth Transfer 42 Days Old.		Sixth Transfer 50 Days Old.		Seventh Transfer 58 Days Old.		Eighth Transfer 62 Days Old.		Totals.	
	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.
202A_2.	56	68	112	110	105	111	51	40											324	329
203A_2.	93	92	110	128	60	89	126	124	172	152	49	60							610	645
203A_3.	156	162	135	150	58	60	78	89	136	123	91	93							654	677
204A_1.	232	194	140	146	20	24													392	364
204A_2.	223	234	155	156	157	151	115	115											650	656
204A_3.	134	128	46	70	38	37													218	235
205A_2.	214	202	109	114	86	104	115	110	12	12									536	542
205A_3.	187	161	140	130	103	113	114	107											544	511
206A_1.	68	58	80	55	75	77	78	76	94	89	72	95	126	118	45	45	10	5	648	618
206A_2.	149	152	173	176	79	126	135	106	152	130	34	23							722	713
206A_3.	173	164	179	207	141	134	129	162	156	159	183	203	71	91					1032	1120
207A_1.	90	87	153	143	81	90	100	100											424	420
207A_2.	120	118	129	123	86	83	84	69											419	393

Selection Experiment I. (Bloomington Stock.)

From a stock of flies collected in Bloomington, a virgin female was selected and mated to a male from the same stock. From the offspring of this pair of flies the high and low strains were started. Before selection was begun, 2,936 flies were examined for sex. The ratio was 1,456 males to 1,480 females, or 1 male to 1.06 females. The ratio here is stated in just the reverse order from the customary form but since Moenkhaus used the reverse order, the ratios in the present discussion will be stated likewise to make the two experiments more comparable. Table II. gives the history of the origin of the high and low strains.

Table II.[1]

Showing Origin of the "High" and "Low" Strains of the Bloomington Stock.


206 (Original pair)
206 A1
206 A2
206 A3
68–58
149–152
173–164
206 B1
206 B2
185–176
175–166
206 C3
206 C4
206 C5
206 C7
206 C8
206 C11
206 C12
15–30
84–74
63–52
Low
108–130
93–100
152–140
91–121
High

Tables III. and IV. give the history of the high and low strains. There is no indication of the ratios having changed in the direction of the selection. In fact, in the high strain where there was selection for a relatively high number of females, the totals show a relatively lower number of females than in the low strain. The totals for the high strain were 8,837 males to 8,942 females or a ratio of 1 male to 1.012 females while in the low strain there were 8,368 males to 9,091 females or a ratio of 1 male to 1.086 females.

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[1] In all tables where two numbers are expressed with a dash between them, as 68–58, the number to the left of the dash always represents the male count and the number to the right, the female count.

TABLE III.

Showing Summary of the "High" Strain of the Bloomington Stock. Experiment I.

Generation.											Totals.
A	91 ♂–121 ♀ (*1 : 1.33*) (From Mating 206C_{12})										
B	2 145–150 *1 : 1.03*	4 200–224 *1 : 1.12*	5 216–229 *1 : 1.06*	6 188–198 *1 : 1.05*	7 179–177 *1 : .99*	8 229–183 *1 : .80*	9 154–162 *1 : 1.05*	10 99–85 *1 : .85*	11 111–116 *1 : 1.05*	13 121–140 *1 : 1.16*	1642–1664 *1 : 1.013*
C	1 78–65 *1 : .83*	3 106–118 *1 : 1.11*	4 153–148 *1 : .97*	5 167–179 *1 : 1.07*	6 53–53 *1 : 1*	8 176–165 *1 : .94*	10 143–136 *1 : .95*	12 127–115 *1 : .91*		13 42–69 *1 : 1.64*	1045–1048 *1 : 1.003*
D	2 201–233 *1 : 1.16*	3 83–83 *1 : 1.0*	5 115–110 *1 : .96*	6 92–90 *1 : .99*	7 221–204 *1 : .92*	9 117–125 *1 : 1.07*	10 95–104 *1 : 1.09*	11 37–35 *1 : .95*	12 139–160 *1 : 1.15*	14 284–254 *1 : .89*	1384–1399 *1 : 1.01*
E	1 117–99 *1 : .85*	2 184–176 *1 : .96*	3 223–225 *1 : 1.01*	4 194–173 *1 : .89*	5 302–279 *1 : .92*	6 179–180 *1 : 1.01*	7 252–278 *1 : 1.1*	10 93–111 *1 : 1.19*	12 195–194 *1 : .99*	13 36–31 *1 : .86*	1775–1746 *1 : .984*
F	1 179–177 *1 : .988*	2 208–188 *1 : .90*	3 79–70 *1 : .89*	4 98–128 *1 : 1.31*	5 188–154 *1 : .82*	7 124–142 *1 : 1.15*	9 149–159 *1 : 1.07*	12 158–154 *1 : .97*	13 113–139 *1 : 1.241*	14 52–61 *1 : 1.18*	1348–1372 1 : *1.018*
G	1 85–96 *1 : 1.13*	2 166–168 *1 : 1.01*	5 31–55 *1 : 1.77*	6 164–174 *1 : 1.06*	8 219–223 *1 : 1.02*	10 125–135 *1 : 1.08*	11 144–143 *1 : .99*	12 70–80 *1 : 1.143*	14 111–119 *1 : 1.07*	15 86–96 *1 : 1.12*	1201–1289 *1 : 1.073*
H	3 95–101 *1 : 1.06*			4 116–103 *1 : .89*		8 64–57 *1 : .89*	9 50–35 *1 : .70*	10 117–128 *1 : 1.09*			442–424 *1 : .959*

Grand Total........8837–8942
1 : 1.012

TABLE IV.

Showing Summary of the "Low" Strain of the Bloomington Stock. Experiment I.

Generation.											Totals.
A	63 ♂–52 ♀ (*1 : .83*) (From Mating 206C_5)										
	1	3	4	5	7	8	9	10			
B	164–228	227–248	109–113	138–146	190–187	35–88	185–184	191–197			1239–1391
	1 : 1.39	*1 : 1.09*	*1 : 1.04*	*1 : 1.06*	*1 : .98*	*1 : 2.51*	*1 : .99*	*1 : 1.03*			*1 : 1.123*
	1	4	5	7	8	9	10	11			
C	123–135	51–53	291–327	105–119	120–114	60–44	176–181	52–71			978–1044
	1 : 1.1	*1 : 1.04*	*1 : 1.12*	*1 : 1.13*	*1 : .95*	*1 : .73*	*1 : 1.03*	*1 : 1.37*			*1 : 1.067*
	2	6	7	9	10	11	12	13	14	15	
D	175–207	153–128	136–160	130–126	133–158	192–196	85–104	225–261	135–158	181–199	1545–1697
	1 : 1.18	*1 : .84*	*1 : 1.18*	*1 : .97*	*1 : 1.19*	*1 : 1.03*	*1 : 1.22*	*1 : 1.16*	*1 : 1.17*	*1 : 1.2*	*1 : 1.098*
	1	2	4	5	8	9	10	11	12	15	
E	176–141	125–123	138–131	141–135	151–151	139–142	285–277	167–141	193–234	180–180	1695–1655
	1 : .8	*1 : .98*	*1 : .95*	*1 : .96*	*1 : 1*	*1 : 1.02*	*1 : .97*	*1 : .84*	*1 : 1.21*	*1 : 1*	*1 : .976*
	1	2	3	4	6	7	9	11	12	14	
F	275–252	174–167	145–179	115–105	173–190	194–180	164–264	246–266	219–215	181–202	1886–2020
	1 : .92	*1 : .96*	*1 : 1.23*	*1 : .91*	*1 : 1.10*	*1 : .93*	*1 : 1.61*	*1 : 1.08*	*1 : .981*	*1 : 1.11*	*1 : 1.071*
	1		6		9	11	14	15			
G	114–134		84–180		89–83	205–190	37–30	84–132			613–749
	1 : 1.18		*1 : 2.14*		*1 : .93*	*1 : .93*	*1 : .81*	*1 : 1.57*			*1 : 1.221*
	1	4	5	7	8	10	12	13	14	17	
H	52–64	56–72	59–87	48–70	38–47	47–53	8–14	9–9	45–61	50–58	412–535
	1 : 1.23	*1 : 1.29*	*1 : 1.47*	*1 : 1.46*	*1 : 1.24*	*1 : 1.13*	*1 : 1.75*	*1 : 1*	*1 : 1.36*	*1 : 1.16*	*1 : 1.298*

Grand Total........8368–9091
1 : 1.086

SELECTION EXPERIMENT II. (Saratoga Stock.)

In the first experiment, a much larger number of flies were examined than were examined by Moenkhaus. But since opposite results were obtained, a criticism might be offered that the writer, by chance, selected a stock which was not affected by selection. So the second experiment was made upon a new and unrelated stock. This stock was collected at Saratoga, Indiana. Fifty matings were made from among the offspring of a single pair. The extremes of these fifty were used as parents of the high and low strains. The counts of these fifty pairs are given in Table V. There were 21,019 flies examined before selection began and the stock showed a ratio of 1 : 1.0512, or 10, 247 males to 10,772 females.

TABLE V.

Showing the Origin of the "High" and "Low" Strains of the Saratoga Stock.

♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.
264	**235**[1]	202	222	252	241	213	248	179	149
157	165	246	236	157	190	187	168	266	263
208	230	290	280	163	202	241	235	224	234
285	256	192	232	214	237	232	257	109	129
137	160	218	223	176	188	245	205	184	171
173	190	187	217	243	282	75	106	226	206
193	187	251	222	160	189	245	225	195	233
155	168	192	207	200	280	188	171	183	168
252	236	**181**	**307**[2]	212	275	129	175	226	222
288	271	183	187	200	225	235	247	234	220
Grand Total.............................						10,247 ♂'s to 10,772 ♀'s			

The results of the selection in this experiment are given in Tables VI. and VII. In this stock there were greater individual variations from the normal sex-ratios in each direction but they did not breed true. The totals show the high strain to have a higher relative number of females than the low strain. They are as follows: for the high strain, 7,377 males to 8,365 females, or a ratio of 1 : 1.134; for the low strain, 10,923 males to 11,246 females or a ratio of 1 : 1.029. Considering these totals, it might seem that something had been accomplished by selection but when the totals are considered generation by generation the results do not seem so conclusive. If selection has been the factor which

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[1] From this mating originated the "low" strain.

[2] From this mating originated the "high" strain.

TABLE VI.

Showing Summary of the "Low" Strain of the Saratoga Stock. Experiment II.

Generation.											Totals.
A	264 ♂–235 ♀ (1 : .89)										
B	1 146–150 1 : 1.03	2 159–202 1 : 1.27	3 199–152 1 : .76	4 199–213 1 : 1.07	5 131–129 1 : .99	6 83–91 1 : 1.10	7 81–78 1 : .96	8 109–119 1 : 1.09	9 108–100 1 : .926	10 107–121 1 : 1.13	1322–1355 1 : 1.024
C	1 144–161 1 : 1.12	2 135–143 1 : 1.06	3 210–208 1 : .99	4 132–130 1 : .98	5 138–149 1 : 1.08	6 157–138 1 : .88	7 224–251 1 : 1.12		8 45–44 1 : .98	10 90–84 1 : .93	1275–1308 1 : 1.0259
D	1 270–294 1 : 1.09	2 212–242 1 : 1.14	3 174–135 1 : .78	4 193–217 1 : 1.12	5 223–211 1 : .95	6 256–246 1 : .96	7 67–81 1 : 1.21	8 219–215 1 : .98	9 308–308 1 : 1	10 267–263 1 : .985	2189–2212 1 : 1.0105
E	1 137–136 1 : .99	2 106–115 1 : 1.08	3 50–65 1 : 1.3	4 146–169 1 : 1.16	5 115–130 1 : 1.13	6 153–153 1 : 1	7 109–138 1 : 1.27	8 206–188 1 : .91	9 32–39 1 : 1.22	10 160–149 1 : .931	1214–1282 1 : 1.056
F	2 166–176 1 : 1.06		3 199–213 1 : 1.07		4 72–84 1 : 1.17		5 171–210 1 : 1.23	6 62–37 1 : .60			670–720 1 : 1.0746
G	1 43–41 1 : .95	2 101–97 1 : .96	3 130–137 1 : 1.05	4 124–121 1 : .98	5 79–90 1 : 1.14	6 154–170 1 : 1.10	7 90–103 1 : 1.14	8 148–156 1 : 1.05	9 68–67 1 : .985	10 205–213 1 : 1.039	1142–1195 1 : 1.0464
H	1 199–197 1 : .99	2 172–169 1 : .98	3 144–152 1 : 1.06	4 169–179 1 : 1.06	5 151–152 1 : 1.01	6 124–141 1 : 1.14	7 92–101 1 : 1.10	8 210–225 1 : 1.07	9 163–185 1 : 1.135	10 114–107 1 : .938	1538–1608 1 : 1.0455
I	1 184–178 1 : .967	2 182–183 1 : 1.005	3 164–137 1 : .835	4 184–180 1 : .978	5 74–97 1 : 1.31	6 111–110 1 : .991	7 187–216 1 : 1.155	8 136–116 1 : .853	9 177–168 1 : .949	10 174–181 1 : 1.04	1573–1566 1 : .996

Grand Total........10,923–11,246
1 : 1.029

TABLE VII.

Showing Summary of the "High" Strain of the Saratoga Stock. Experiment II.

Generation.											Totals.
A				181 ♂–307 ♀ (*1 : 1.696*)							
B	1 149–163 *1 : 1.09*	2 145–152 *1 : 1.05*	3 147–212 *1 : 1.442*	4 208–194 *1 : .932*	5 201–178 *1 : .885*	6 172–198 *1 : 1.151*	7 143–149 *1 : 1.04*	8 240–240 *1 : 1*	9 22–22 *1 : 1*	10 207–231 *1 : 1.116*	1634–1739 *1 : 1.064*
C	2 72–81 *1 : 1.125*		4 122–109 *1 : .893*		6 163–158 *1 : .969*	8 117–118 *1 : 1.008*	9 106–148 *1 : 1.396*	10 144–136 *1 : .944*			724–750 *1 : 1.036*
D	1 171–192 *1 : 1.123*	2 132–157 *1 : 1.189*	3 119–203 *1 : 1.706*	4 189–324 *1 : 1.714*	5 191–221 *1 : 1.157*	6 175–212 *1 : 1.211*	7 140–222 *1 : 1.586*	8 127–189 *1 : 1.488*	9 97–169 *1 : 1.742*	10 136–149 *1 : 1.096*	1477–2038 *1 : 1.3798*
E	1 107–143 *1 : 1.34*	2 111–180 *1 : 1.62*	3 152–144 *1 : .95*	4 93–109 *1 : 1.17*	5 88–104 *1 : 1.18*	6 207–181 *1 : .874*	7 70–106 *1 : 1.51*	8 168–228 *1 : 1.36*	9 98–99 *1 : 1.01*	10 33–43 *1 : 1.30*	1127–1337 *1 : 1.186*
F	2 258–276 *1 : 1.07*	3 50–37 *1 : .74*	4 118–107 *1 : .907*	5 119–102 *1 : .86*	6 260–221 *1 : .85*	7 81–104 *1 : 1.284*	8 33–28 *1 : .85*	9 163–206 *1 : 1.264*		10 49–56 *1 : 1.14*	1131–1137 *1 : 1.0053*
G	1 33–84 *1 : 2.55*	2 82–111 *1 : 1.35*	3 152–138 *1 : .91*	4 32–41 *1 : 1.28*	5 78–94 *1 : 1.21*	7 42–38 *1 : .88*	8 27–30 *1 : 1.11*	9 89–91 *1 : 1.022*			536–627 *1 : 1.1698*
H	2 74–79 *1 : 1.068*		3 154–151 *1 : .9805*	4 108–129 *1 : 1.19*	5 40–28 *1 : .70*	6 99–89 *1 : .899*		9 37–42 *1 : 1.135*			512–518 *1 : 1.0117*
I	1 63–81 *1 : 1.28*		3 22–15 *1 : .68*		4 60–56 *1 : .93*	6 68–49 *1 : .72*		8 23–18 *1 : .78*			236–219 *1 : .928*

Grand Total........7,377–8,365
1 : 1.134

has made the difference between the two strains, the difference should be most evident in the later generations of the experiment. This is not true, for a moment's calculation will show that the last three generations in each strain do not show as extreme an average ratio in the desired direction as those which preceed tehm. Although the difference between the totals of the two strains may be sufficiently large to be considered significant, it seems more probable that it is a chance variation, especially since the first experiment showed a similar variation in the direction opposite selection.

Selection Experiment III. (Warsaw Stock.)

In order to make the work still more conclusive, selection was attempted upon a third stock. This stock was collected at Warsaw, Indiana, and kept as a stock culture in the laboratory for about six months before the experiment was started.

Here forty-two pairs were mated from among the offspring of a single pair and the extremes of these matings were used as the parents of the high and low strains.[3] There were 11,190 flies examined before the selection began, 5,448 males to 5,742 females, a ratio of 1 : 1.0539. The counts of these matings are given in Table VIII.

Table VIII.

Showing Origin of the "High" and "Low" Strains of the Warsaw Stock.

♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.
38	43	91	82	125	106	226	220	255	247
66	72	106	101	155	150	110	114	136	143
133	169	135	156	**235**	**238**[2]	80	72	84	77
192	208	41	39	102	105	74	93	94	100
85	75	105	128	48	61	114	129	187	200
147	**187**[1]	178	189	186	186	195	203	89	79
170	192	91	102	127	142	179	199	175	171
105	113	101	104	187	220	158	156		
125	119	106	114	112	138				
Grand Total . . . . 5,448 ♂'s to 5,742 ♀'s									

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[1] From this mating originated the "high" strain.

[2] From this mating originated the "low" strain.

[3] It will be seen in Table VIII. that the pair used as the parents of the low strain did not produce the most extreme ratio in that direction. This mistake was due to a mathematical error which was not checked up until it was too late to rectify the experiment.

In the latter part of this experiment there was a diveation from the usual methods. The element of time was sacrificed to be able to make a larger number of matings in each generation. A number of males and females were isolated from each mating, being examined, and held to be mated after all of the counts for the generation were finished. By this method it was necessary to make matings only from the cultures which had given the most desirable ratios but in so doing a considerable amount of time was lost between each generation.

This third attempt to modify, by selection, the sex-ratio did not result in bringing about any very significant difference between the two strains. (Tables IX. and X. give the results of selection upon this stock.) The ratios computed from the totals in each strain showed a slight difference in the desired direction but this was so slight that it might be due to chance. Therefore it seems that no definite conclusions can be drawn from this experiment. In the low strain the totals were 9,673 males to 9,951 females, or a ratio of 1 : 1.0287 and in the high strain 12,327 males to 12,898 females, or a ratio of 1 : 1.0463.

CONCLUSION.

Since three distinct and unrelated stocks of flies show no significant effect of selection, it seems safe to conclude from these data that Moenkhaus's conclusion concerning the amenability of the sex-ratio in *Drosophila ampelophila* will not hold.

DISCUSSION OF MOENKHAUS'S WORK.

Moenkhaus concludes from his work on the effect of selection on sex-ratios in *Drosophila ampelophila* that sex-ratios are "strongly transmissible and amenable to the process of selection." From his paper it is somewhat difficult to tell whether Moenkhaus intended to say that he had developed by selection, strains characterized by high and low female ratios or that by selection, he simply maintained a high and low strain which he found in nature. His results are given in the discussion which follows.

By way of explanation it might be said that Moenkhaus called the strain in which he selected for a relatively higher female ratio the "female" strain, and the one in which he selected in

TABLE IX.

Showing Summary of the "Low" Strain of the Warsaw Stock. Experiment III.

Generation.													Totals.
A	235 ♂s–238 ♀s (*1 : 1.013*)												
B	2 183–171 *1 : .934*	3 266–256 *1 : .962*	4 141–144 *1 : 1.02*	5 150–155 *1 : 1.033*	6 112–139 *1 : 1.24*	7 227–216 *1 : .951*	9 224–254 *1 : 1.13*	10 155–125 *1 : .806*					1458–1460 *1 : 1.0013*
C	1 222–207 *1 : .932*	2 234–252 *1 : 1.077*	3 231–242 *1 : 1.048*	4 105–112 *1 : 1.067*	5 211–254 *1 : 1.204*	6 164–193 *1 : 1.177*	7 247–246 *1 : .996*	8 158–160 *1 : 1.013*	9 96–115 *1 : 1.198*	10 111–109 *1 : .982*			1779–1890 *1 : 1.062*
D	1 163–151 *1 : .926*	2 226–238 *1 : 1.053*	3 141–153 *1 : 1.085*	4 139–125 *1 : .899*	5 206–227 *1 : 1.10*	6 204–209 *1 : 1.025*	7 200–297 *1 : 1.49*	8 142–177 *1 : 1.246*	9 193–187 *1 : .969*	10 188–196 *1 : 1.04*			1802–1960 *1 : 1.087*
E	1 51–46 *1 : .902*	2 96–111 *1 : 1.156*	3 37–45 *1 : 1.216*	4 125–119 *1 : .952*	5 84–89 *1 : 1.059*	6 56–48 *1 : .857*	7 101–101 *1 : 1*	8 73–68 *1 : .93*					
E	9 88–92 *1 : 1.045*	10 101–112 *1 : 1.109*	11 86–78 *1 : .907*	12 88–86 *1 : .977*	13 71–94 *1 : 1.324*	14 63–84 *1 : 1.33*	15 53–62 *1 : 1.169*						1173–1235 *1 : 1.053*
F	1 151–123 *1 : .815*	3 100–72 *1 : .72*	4 192–158 *1 : .823*	5 135–152 *1 : 1.126*	6 113–96 *1 : .849*	7 121–110 *1 : .909*	8 117–129 *1 : 1.102*	9 41–32 *1 : .78*	10 97–80 *1 : .83*	11 99–123 *1 : 1.242*			
F	12 95–123 *1 : 1.295*	13 154–153 *1 : .99*	14 44–33 *1 : .75*	15 68–96 *1 : 1.41*	16 209–202 *1 : .966*	17 59–57 *1 : .97*	18 169–189 *1 : 1.118*	19 70–73 *1 : 1.043*	20 55–52 *1 : .945*	21 51–43 *1 : .843*			2140–2096 *1 : .979*
G	1 64–64 *1 : 1*	2 103–97 *1 : .94*	4 51–72 *1 : 1.41*	7 25–27 *1 : 1.08*	9 28–38 *1 : 1.36*	10 39–26 *1 : .67*	12 59–79 *1 : 1.34*	13 36–34 *1 : .94*	15 42–27 *1 : .64*	16 79–83 *1 : 1.05*	18 33–39 *1 : 1.18*	19 34–22 *1 : .647*	
G	20 19–37 *1 : 1.95*	21 55–46 *1 : .84*	22 29–29 *1 : 1*	23 88–78 *1 : .89*	24 34–34 *1 : 1*	25 89–69 *1 : .78*	26 104–108 *1 : 1.04*	27 75–79 *1 : 1.05*	28 65–71 *1 : 1.09*	30 115–112 *1 : .974*	31 55–39 *1 : .709*		1321–1310 *1 : .9916*

Grand Total........9673–9951
1 : 1.0287

TABLE X.

Showing Summary of the "High" Strain of the Warsaw Stock. Experiment III.

Generation.											Totals.
A					147 ♂s–187 ♀s (*1 : 1.272*)						
	1	2	3	4	5	6	7	8	9	10	
B	126–115 *1 : .913*	195–210 *1 : 1.08*	52–42 *1 : .81*	53–76 *1 : 1.43*	119–127 *1 : 1.07*	138–143 *1 : 1.04*	191–188 *1 : .98*	194–196 *1 : 1.01*	206–189 *1 : .92*	208–253 *1 : 1.22*	1482–1539 *1 : 1.039*
	1	2	3	4	5	6	7	8	9	10	
C	76–95 *1 : 1.25*	168–202 *1 : 1.20*	209–204 *1 : .98*	80–85 *1 : 1.06*	228–242 *1 : 1.06*	59–66 *1 : 1.12*	154–170 *1 : 1.10*	183–176 *1 : .962*	160–179 *1 : 1.12*	181–149 *1 : .82*	1498–1568 *1 : 1.047*
	1	2	3	4	5	6	7	8	9	10	
D	43–43 *1 : 1*	69–78 *1 : 1.13*	79–90 *1 : 1.14*	141–152 *1 : 1.08*	149–141 *1 : .946*	147–139 *1 : .946*	194–190 *1 : .979*	195–202 *1 : 1.04*	193–181 *1 : .94*	146–152 *1 : 1.04*	1356–1368 *1 : 1.009*
	1	2	3	4	5	6	7	8			
E	28–39 *1 : 1.39*	36–41 *1 : 1.14*	74–93 *1 : 1.26*	59–70 *1 : 1.19*	112–116 *1 : 1.04*	123–128 *1 : 1.04*	107–121 *1 : 1.13*	94–91 *1 : .97*			
	9	10	11	12	13						
E	92–105 *1 : 1.14*	52–62 *1 : 1.19*	84–82 *1 : .976*	67–121 *1 : 1.806*	105–126 *1 : 1.2*						1033–1195 *1 : 1.157*
	1	2	3	4	5	6	7	8	9	10	
F	145–146 *1 : 1.007*	214–245 *1 : 1.15*	249–246 *1 : .988*	28–65 *1 : 2.32*	168–179 *1 : 1.07*	169–162 *1 : .96*	154–162 *1 : 1.05*	77–63 *1 : .82*	133–146 *1 : 1.098*	79–74 *1 : .94*	

TABLE X.—*Continued.*

	11	12	13	14	15	16	17	18	19	20		
F	100–102 *1 : 1.02*	214–228 *1 : 1.07*	195–226 *1 : 1.16*	182–199 *1 : 1.09*	177–149 *1 : .842*	208–214 *1 : 1.03*	194–207 *1 : 1.07*	121–135 *1 : 1.12*	148–157 *1 : 1.06*	93–86 *1 : .93*		
	21	22	23	24	25	26	27	28				
F	132–127 *1 : .96*	75–98 *1 : 1.31*	115–149 *1 : 1.3*	114–122 *1 : 1.07*	192–206 *1 : 1.07*	113–130 *1 : 1.15*	118–136 *1 : 1.15*	173–156 *1 : .902*				4080–4315 *1 : 1.0576*
	1	2	3	4	5	6	7	9	10	11	12	
G	57–69 *1 : 1.21*	58–56 *1 : .97*	85–91 *1 : 1.07*	49–56 *1 : 1.14*	33–27 *1 : .82*	84–72 *1 : .86*	26–46 *1 : 1.77*	44–49 *1 : 1.11*	90–94 *1 : 1.04*	112–122 *1 : 1.09*	92–83 *1 : .90*	
	13	14	15	16	17	18	19	20	21	22	23	
G	65–67 *1 : 1.03*	46–51 *1 : 1.11*	128–109 *1 : .85*	73–75 *1 : 1.03*	53–49 *1 : .93*	73–86 *1 : 1.18*	41–44 *1 : 1.08*	62–49 *1 : .79*	34–32 *1 : .94*	68–76 *1 : 1.12*	63–98 *1 : 1.56*	
	24	25	26	27	28	29	30	31	32	33	34	
G	36–27 *1 : .75*	39–60 *1 : 1.54*	80–69 *1 : .86*	53–33 *1 : .62*	52–62 *1 : 1.19*	134–108 *1 : .81*	42–35 *1 : .83*	50–48 *1 : .96*	49–39 *1 : .796*	103–113 *1 : 1.097*	71–87 *1 : 1.23*	
	35	36	37	38	39	40	41	42	43	44	45	
G	113–109 *1 : .97*	58–57 *1 : .98*	84–94 *1 : 1.12*	32–27 *1 : .84*	29–32 *1 : 1.10*	60–50 *1 : .83*	61–64 *1 : 1.05*	27–36 *1 : 1.33*	67–70 *1 : 1.05*	152–126 *1 : .829*	50–66 *1 : 1.32*	2878–2913 *1 : 1.0122*

Grand Total........12,327–12,898
1 : 1.0463

the opposite direction the "male" strain. This terminology will be used throughout this discussion. As the starting point of his female strain, he selected from nature a pair of flies which produced 52 males and 135 females and in his male strain he started with a pair which produced 84 males and 75 females.

Considering first the effect of selection upon his female strain we find here no conclusive evidence of any progressive change in the direction of the selection. (His data for this strain are given in Table XI.) To be sure, there is a slight increase in the relative number of females in his second and third generations over his first generation, but this fluctuation could easily be attributed to the small number of individuals examined. The greatest difficulty in assuming the effectiveness of selection here, is the fact that his last two generations are the ones in which he obtained the lowest relative number of females. Moenkhaus explains these low ratios of the last two generations in that he possibly made a poor selection in the preceding generation, but if we consider selection as having a cumulative effect (as Moenkhaus seems to consider it) it is difficult to see how one could lose by one poor selection all that he had accomplished in the previous selections. It must be admitted that by using as parents of each succeeding generation, pairs which threw the most extreme ratios in favor of the females, Moenkhaus was able to maintain a strain which on an average gave a relatively high number of females. But this was probably not due to any cumulative effect of selection but to the isolation of a peculiar type of female which will be discussed more fully later.

As to the male strain (Table XII)., Moenkhaus admits that the effect of his selection here has been slight and after examining his data carefully it is difficult to see how it could be assumed that there has been even a slight effect of selection. He started with a ratio (84 males to 75 females) which was only slightly different from normal and this slight difference was not transmitted to the first generation nor any succeeding one. So it seems that he had here a normal strain which would have given the same ratio regardless of the direction of the selection.

The female strain is the unusual one and is in need of an explanation. It throws some very exceptional ratios in favor of

TABLE XI.

History of Moenkhaus's "Female" Strain.

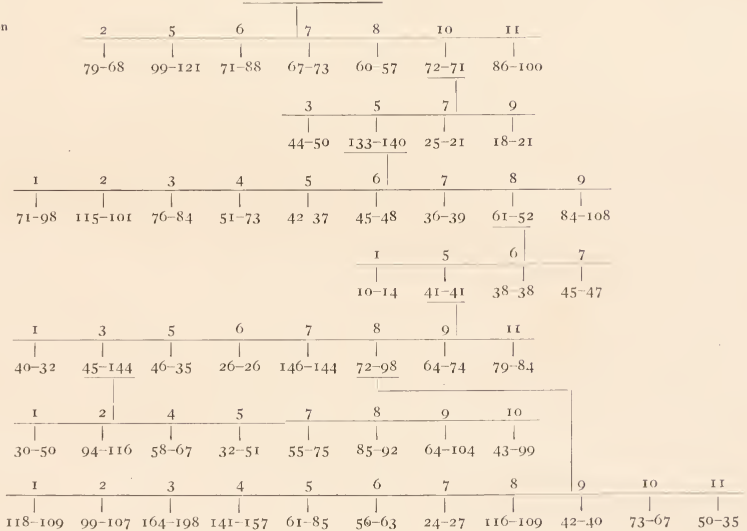
206 (*52* ♂s : *135* ♀s)

Generation.												Totals.
1	1 21–37	2 42–68	3 71–164	4 76–71	5 108–125	6 51–80	7 43–67	8 51–46	9 53–103	10 8–56	11 55–102	578–969 *1 : 1.67*
2						7 21–49	8 41–94	9 55–70	10 98–178			215–391 *1 : 1.82*
3				3 15–37	4 18–54	5 25–37	7 35–71					93–201 *1 : 2.17*
4	1 26–47	2 39–56	3 33–52	5 64–67	7 53–93	8 85–162	10 54–106					354–483 *1 : 1.36*
5					4 121–139	5 32–32	6 93–101	7 40–114	8 86–132			372–518 *1 : 1.39*
5	2 51–44	3 67–53	5 41–47	6 74–78	7 120–130	10 102–136	13 41–47					496–535 *1 : 1.07*

TABLE XII.

History of Moenkhaus's "Male" Strain.

207 (84 ♂s : 75 ♀s)



Generation	1	2	3	4	5	6	7	8	9	10	11	Totals.
1		79–68			99–121	71–88	67–73	60–57		72–71	86–100	536–579 *1 : 1.08*
2			44–50		133–140		25–21		18–21			220–223 *1 : 1.00*
3	71–98	115–101	76–84	51–73	42 37	45–48	36–39	61–52	84–108			581–640 *1 : 1.10*
4	10–14				41–41	38–38	45–47					142–147 *1 : 1.04*
5	40–32		45–144		46–35	26–26	146–144	72–98	64–74		79–84	518–607 *1 : 1.17*
6	30–50	94–116		58–67	32–51		55–75	85–92	64–104	43–99		561–654 *1 : 1.42*
6	118–109	99–107	164–198	141–157	61–85	56–63	24–27	116–109	42–40	73–67	50–35	944–997 *1 : 1.05*

the females and these exceptional individual ratios caused the relative number of females in the female strain to be higher than normal. These unusual ratios can be readily explained by assuming that the female which Moenkhaus selected as the mother of his female strain carried a recessive sex-linked lethal factor and it will be pointed out later that all of Moenkhaus's data substantiate, very precisely, this assumption. The existence of such factors has been conclusively demonstrated by the recent work of Morgan and his students. A recessive lethal factor is any factor that brings about the death of the individual in which it occurs, provided its effect is not counteracted by the action of its normal allelomorph. Then if a lethal is sex-linked, all males which get it will die for they cannot carry its normal allelomorph since they possess but one X-chromosome. Since all males receiving the lethal factor die, this factor is never transmitted by the male and as a consequence the female can never be homozygous for it. Therefore the lethal factor has no effect on the female but she, by transmitting it to half of her sons, causes their death. Since half of the males die, a 2:1 ratio will result. A female carrying a lethal will transmit it to half of her daughters and they will always be heterozygous for it since they cannot receive it from their father.

Then the female which Moenkhaus used as the mother of his female line was probably a lethal female. It should be said that Moenkhaus is not to be criticised for not considering lethals in his paper, for lethals were not known till two years after his work was published. The original female gave a ratio which was an approximate 2:1 ratio. As mothers of the succeeding generations, Moenkhaus probably selected lethal bearing females (excluding female 5 of generation 4). These females should transmit the lethal factor to half their daughters and this expectation is realized, for from the 31 matings made in the female strain (excluding the offspring of female 5 of generation 4), fifteen 2:1 ratios resulted. Those considered 2:1 ratios are matings 3, 9 and 11 of generation 1; 7, 8 and 10 of generation 2; 3, 4 and 7 of generation 3; 1, 7, 8, and 10 of generation 4; and 7 and 8 of generation 5. So the number of 2:1 ratios obtained would justify the assumption of the presence of a lethal factor.

Another indication of the presence of a lethal factor is the fact that in generation 4, when he used female 5 which gave a normal ratio, as mother of the next generation, all of her daughters also threw normal ratios. This female did not carry the lethal factor and none of her offspring showed abnormal ratios.

A third indication of a lethal factor is the fact that Moenkhaus found in crossing his male and female strains that the female is almost wholly responsible for the transmission of the sex-ratio. For he found if females from a strain possessing a high female ratio be mated with males from a strain possessing a low female ratio or vice versa, the offspring will show a sex-ratio which is wholly or very near that of the strain from which the females were taken. If his female strain carried a lethal factor and if his male strain was a normal strain, as it appears to be, the above result would be the expected one. For, if a lethal bearing female is mated to a normal male, she will transmit the lethal factor to half her daughters but a male from the lethal strain cannot transmit the lethal factor because all of the lethal bearing males die. Therefore the end result would be as Moenkhaus found, that the offspring will show sex-ratios like that of the strains from which the females came.

Of course, the only sure way of testing for a sex-linked lethal factor is to cross the suspected female to a male carrying another sex-linked factor and the resulting F_2 will be characterized by a deficiency in the class of normal males. So it is not possible to determine beyond doubt whether the unusual ratios in Moenkhaus's female strain were due to a lethal factor. But since several lethals have already been found and all of his data substantiate this assumption, it seems that this explanation is probably the correct one.

Discussion.

The writer's three attempts to modify by selection, the sex-ratio in the fruit fly have resulted in no clear-cut evidence that it was modifiable. In each experiment, the work covered more generations and many more individuals than the work of Moenkhaus. Since Moenkhaus's results could not be obtained in any one of the three experiments, it leads one to question Moenkhaus's conclusions or, at least, to question the general application of his findings. The fact that the difference which Moenkhaus found between his two strains can be readily explained to be due to a cause entirely independent of the cumulative effect of selection, makes his work support, rather than oppose the findings of the writer. It must be admitted that negative results in secletion work can never be conclusive, for the criticism may always be offered that selection over a greater period might have yielded different results. But, nevertheless, we feel justified in concluding from the data here presented that the sex-ratio in *Drosophila ampelophila* is not readily, if at all, modifiable by selection.

LETHALS.

Since the offspring of over 700 pairs of flies were examined for sex in these experiments, and since extreme ratios were sought, it would not be surprising to find a sex-linked lethal mutation. In fact, there is fairly good evidence that one such mutation occurred. This was mating number of 6 generation G of the low strain of the Bloomington stock (Table IV.). This gave an approximate 2:1 ratio (84 males to 180 females). Four matings were made from among the offspring of this mating and three out of the four gave 2 to 1 ratios while the fourth was doubtful. If a sex-linked lethal mutation had occurred, half of the four should have given 2 to 1 ratios and the other half normal. Since so few matings were made, it is possible that all the females chosen were lethal bearers. The stock was lost by accident at this time and further tests were not possible. But since the unusual ratio was transmitted it is probable that a mutation occurred here. In the high series of the Saratoga stock, there were several ratios which approximate a 2 to 1 ratio but none of the flies showing it transmitted this tendency to their offspring. Also in the high strain of the Warsaw stock, generation E mating 12 gave a 2 to 1 ratio. A large number of matings were made among the offspring of this pair but this unusual ratio was not transmitted to any of the progeny.

In all of the matings examined there were comparatively few ratios found which were two to one or more.[1] These extreme ratios were more frequently in favor of the females and in only one case was there any evidence that a sex-linked lethal mutation had occurred.

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[1] The unusual ratios more frequently occurred where the counts were very small. These cases were considered to have been due to some unfavorable environmental condition.

In conclusion, I wish to express my thanks to Professor Fernandus Payne for the suggestion of this problem and also for many helpful suggestions and criticism while the work was in progress. I am also greatly indebted to my wife, Elmira Shierling Warren, for help, both in the carrying out of the experiment and in the preparation of the manuscript.

SUMMARY.

1. The sex-ratio in *Drosophila ampelophila* is 100 females to 95 males.

2. The age of the parent has no effect upon the sex-ratio of its offspring.

3. The difference which Moenkhaus found between his two strains, and which he attributed to selection, was probably due to the fact that his male strain was a normal one and his female strain was a lethal bearing one.

4. The sex-ratio in *Drosophila ampelophila* is not readily, if at all, modifiable by selection.

5. There was probably one sex-linked lethal mutation in the writer's selection stocks.

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EFFECTS OF AGING UPON GERM CELLS AND UPON EARLY DEVELOPMENT.

Part II., Changes in Moderately Aged Eggs and Sperm.

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In a previous publication (Goldfarb, '17), the writer determined the variation of freshly liberated sperm and eggs from freshly collected sea urchins of three different species. Two of these, *Toxopneustes* and *Hipponoë*, inhabit the shallow tropical waters of the Dry Tortugas, and the third, *Arbacia*, is common in the deeper colder waters near Woods Hole, Mass. In each species preliminary experiments had been made to ascertain the optimum conditions of development, such as volume and surface and filtration of sea water, concentration of eggs and sperm, etc., and such optimum conditions were used in all subsequent experimentation.

It was shown that even under these constant and optimum conditions, the freshly liberated eggs of different females varied in respect to (1) size, (2) jelly layer, (3) membrane formation, and (4) cleavage; that there was a surprisingly large variation, with respect to each of these four classes, in the eggs of different females; that the sperm of different males also varied, but to a much less degree. And finally the range of variation was determined for each species.

It was suggested that these variations served as convenient and accurate indices of the physiologic condition or vitality of the freshly removed eggs or sperm of different individuals.

In the present paper the same technique, the same three species of sea urchin, the same nomenclature and the same precautions were taken. It is proposed to determine first the physiologic condition of the germ cells, at the time of liberation, from given individuals, and then to determine the nature and the extent of the changes in the eggs and sperm as they become increasingly "overripe," "aged" or" stale."[1] In the next study, Part III., it is proposed to study other changes that appear in very late stages of overripening or ageing of the germ cells. I reserve for Part III. a discussion of the entire problem, and the bibliography.

The changes that were observed in moderately aging eggs, and those which will be discussed in detail are: (1) size, (2) jelly layer, (3) membrane formation and (4) cleavage.

I wish to acknowledge my indebtedness to Dr. A. G. Mayer, Director of the Marine Biological Laboratory of the Carnegie Institution of Washington, and to Prof. F. R. Lillie, Director of the Marine Biological Laboratory at Woods Hole, Mass., for the opportunity of working on this problem at their respective laboratories, and for the many facilities offered in connection with this work.

EFFECT OF AGE UPON SIZE OF EGGS.

Hipponoë and Arbacia.

The diameters of freshly removed unfertilized eggs of each freshly collected female, were measured with an ocular micrometer (no. 3 eye piece and ⅙ objective) and the usual precautions concerning pressure, focusing, sampling, etc., taken. At different intervals (ages) other samples of the eggs of the same female were measured. The data are brought together in Table I.

I have shown (Goldfarb, '17) that the normal size of freshly liberated eggs of *Hipponoë* varied from 18½ to 20 ocular units in diameter, with a mode of 19½ units. In the present series

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[1] By age is meant the time since removal or extrusion of ripe germ cells.

TABLE I.

SHOWS CHANGE IN SIZE AND LOSS OF JELLY LAYER WITH AGE. SIZE IS GIVEN IN OCULAR MICROMETER UNITS OF THE DIAMETER OF THE EGGS AT DIFFERENT AGES. *Hipponoë.*

No.	Date.	Age of Eggs, Hrs.	Diameter of Eggs in Ocular Micrometer Units.									Per Cent. of Eggs with Jelly Layer.	Per Cent. Loss of Jelly per Hour.
			21.0.	20.5.	20.0.	19.5.	19.0.	18.5.	18.0.	17.5.	17.0.		
			Number of Eggs.										
1	6/15	5				*	*						
		22	*	*	*								
2	6/23	5				1	8	1				97	
		29				4	7	1				78	0.7
3	6/28	½				1	7	4				100	
		18½				8	18	3				100	0.0
4	6/28	½			2	5	6	2				100	
		18½			3	8	5	0				94	0.3
5	6/25a	2			2	7	1					100	
		5		2	8	3	1					91	
		24		7	1	3	2					40	2.7
6	6/27a	3			1	12	3					96	
		9			12	5	1					96	
		27		1	9	2	0					79	0.7
		33		0	13	3	0						
7	6/26a	0		1	3	6	3					86	
		18		3	3	6	0					77	
		24				9	5	1				62	1.0
		40						1	9	6	5	0	
		47							1	9	2	0	
8	6/26b	0			8	3						95	
		18	2	4	8	4						95	
		24			7	3						72	0.9
9	6/26c	0				0	4	8				90	
		18				0	15	2				60	
		24				3	8	3				6	3.5
		40				3	12	1				2	
		47				1	6	6				1	
10	6/25b	2				1	9	1				63	
		5				1	10	1				1	
		24					5	7	4	1		0	2.8
11	6/27b	3	2	4	3	6	0	0				80	
		9			1	4	9	1				10	
		27			1	9	2	0				1	3.3
		33			1	2	3	0	2	2	1	0	
12	6/26c	0			5	10						99	
		18		2	12	2						98	
		24		0	12	1	1					98	0.4
		40		1	11	1	1					62	
		47		1	12	1	0					17	
Variation in freshly liberated eggs of different females...			2	6	34	72	54	16	0	0	0	90	

the same variations occurred in the freshly liberated germ cells. As the eggs aged, there was a clear, considerable and progressive change in size. The observations may best be considered in three groups.

Female 1, is an example of the first group. When her eggs were 5 hours old, *i. e.*, 5 hours after liberation, their diameters were 19 and 19.5 units. When her eggs were 22 hours old, they measured 20, 20.5 and 21 units, *a clear and sharp increase in minimum, mode and maximum diameters, with age.*

In female 2, observations were made when the eggs were 5 and 29 hours old. In this female there was also an enlargement but very small. In females 3 and 4, observations were made at ½ and 18 hours respectively. In each female the eggs enlarged with age. In female 5, three observations were made, viz., 2, 5 and 24 hours, with a progressive enlargement at each successive age. In female 6, observations were made at four intervals, 3, 9, 27 and 33 hours. As in female 5, there was at first a rapid enlargement of the eggs, then a slow increase, and, in very late aging, a rapid increase again. The average diameters at each of the four intervals was 19.44, 19.80, 19.77 and 19.96.

In all the eggs of this first group, there was a cumulative increase in volume, with age, for the first 33 hours.

The second group includes such females whose eggs were observed over a longer period than 33 hours. In this group as in the first, the eggs also enlarged at first, with age, but after a longer interval there occurred a secondary diminution in size, and in very old eggs became even smaller than the norm.

For example, the eggs of female 7 were measured at 0, 18, 24, 40 and 47 hours. When 18 hours old the eggs were slightly larger than at the first observation. At 24 hours, the eggs were clearly smaller, and at 40 hours smaller still. At 47 hours they were much below their original size and below the norm for the species. The maximum size had diminished from 20.5 to 18, the mode from 19.5 to 17.5, the minimum from 19 to 17.

In female 8, there was the same initial increase and subsequent diminution towards the norm, when 24 hours old. In female 9, observations were made at 0, 18, 24, 40 and 47 hours. There occurred the usual enlargement until the 40th hour, after which there was a definite diminution in volume.

In a third group, of a few females only, the eggs though freshly removed, were in poor physiologic condition. In this group *the initial enlargement did not take place.* In these eggs *there occurred a direct diminution with age.* In this group belong females 10 and 11, and probably female 7.

It should be recalled that the physiologic condition of any set of eggs was not determined merely by egg size, but by the results of several tests, such as the jelly layer test, the membrane test as well as the cleavage test, etc. By all of these tests, it was clearly and definitely shown *that eggs in good physiologic condition at the time of liberation, increased steadily with age, within the limits shown in Table I., and subsequently diminished in size by a process of fragmentation described in Part III. Eggs in physiologically poor condition at the time of liberation behaved like half-aged eggs, that is, they either enlarged but little, or did not enlarge at all, and subsequently diminished to and below the norm of the species.*[1]

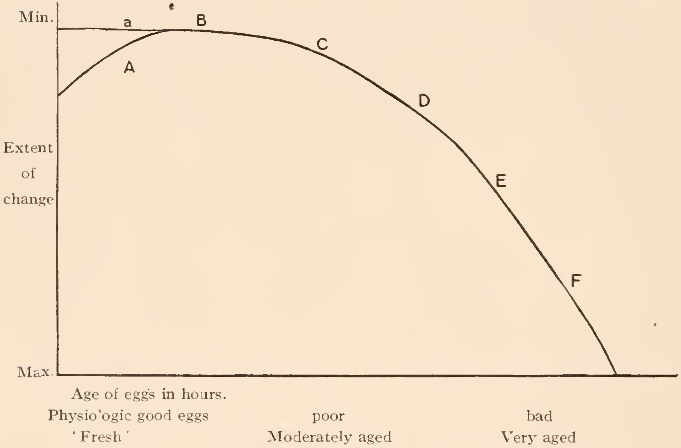

FIG. 1. Extent of morphologic and physiologic change in aging or deteriorating eggs. *A′, B, C, D, E, F,* represents the change in volume, rate of membrane formation, rate of cleavage, total cleavage (*Hipponoë*). *A, B, C, D, E, F,* represents the change in width of fertilization membrane, loss of jelly, total cleavage, etc. *A′, B* is period of superripening; *B, C, D,* the period of overripening or deterioration; *D, E, F,* the period of extreme deterioration or dying of the eggs.

Figure 1 represents graphically and schematically the change in volume with age. Physiologically good eggs show an initial rapid increment, region *A* of the curve; then a progressively slower increment, region *B;* thirdly a progressive *diminution*, region *C*, towards *D* or even below the norm of the species to *E*. Eggs in moderately poor physiologic condition at liberation have already undergone physiologic deterioration, corresponding with part *A* within the body of the mother. Upon liberation they show only parts *B*, *C*, *D*, etc., of the curve. Eggs in very poor condition upon liberation have undergone changes represented by parts *A* and *B*, within the body of the mother, and upon liberation show only parts *C* and *D*, etc.

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[1] So constant is the relation between volume and physiologic condition, tha one can predict the one from the other, with remarkable exactness.

The significance of this increment with age lies in the changed permeability of the cortical layer which is denoted by increase in volume. This progressive increase in permeability permits increasing volumes of sea water to penetrate the egg and thus enlarge it. This process continues until partial cytolysis (fragmentation) reduces the size once again towards the norm, or until complete cytolysis destroyed the eggs altogether.

The change in size with age really measures the change in permeability of the cortical layer.

Similar observations were made upon *Arbacia* eggs, and the changes are so much in agreement with those of *Hipponoë* that it seems unnecessary to describe them here. There is the same initial increment followed by the secondary diminution with age. And the rate of change depended upon the physiologic condition of the eggs at the time of liberation and upon temperature. The two species differ in the markedly slower rate of change of *Arbacia*, due in largest part to the lower temperature of the sea water.

Effect of Age Upon the Jelly Layer.

Hipponoë and Arbacia.

The jelly layer of the same sea-urchin eggs were made clearly visible by adding Chinese black to the sea water, as suggested by F. R. Lillie. Samples of 150 to 200 consecutive freshly removed (and unfertilized) eggs of each female were examined, avoiding mechanical or chemical removal of the jelly, and the presence or absence of the jelly layer recorded. At successive intervals other samples of the same female were examined, and the results reduced to percentages as recorded in Table I.

I have shown (Goldfarb, '17) that the freshly liberated eggs of different females vary considerably in the per cent. possessing the jelly layer. In *Hipponoë* the per cents vary from 100 to 63 per cent., in *Arbacia* from 100 to 59 per cent. These differences, I suggested, indicated corresponding differences in physiologic condition of the eggs at the time of liberation.

Whatever the condition of the eggs at the time of liberation, with increasing age there was a continuous decrease in the number of eggs with the jelly layer present.

The data may best be considered in two groups, according to the physiologic condition of the eggs at the time of the first observation.

In the first group are those females whose eggs were in good physiologic condition. This was indicated by the high per cent. (*i. e.*, above 95 per cent. with jelly layers) at the first observation, as well as by other tests. In this group are placed females 2, 3, 4, 5, 6, 8 and 12, and the corresponding percents of jelly layers was 97, 100, 100, 100, 96, 95 and 99 respectively.

In the second group are placed those females whose eggs were in poor physiologic condition. In this group are included females 7, 9, 10 and 11, whose corresponding jelly counts were, 86, 90, 63 and 80 per cent., respectively.

In both groups the surrounding jelly layer progressively disappeared with age, as seen in Table I. But the rate of loss was different. An example of the first group is female 8. When 0 hours old, 95 per cent. possessed the jelly layer, when 18 hours old 95 per cent., and when 24 hours old only 72 per cent. retained the layer. Female 11 is an example of the second group in which the loss was decidedly greater. The eggs when 3 hours old showed 80 per cent. with jelly layers, when 9 hours old but 10 per cent., when 27 hours old only 1 per cent., and when 33 hours old none of the eggs possessed the jelly envelope.

The two groups differ then, not only in original per cent. with jelly layers, but also in the rate of loss of this layer. In the first, containing physiologically good eggs, the loss of the jelly layer per hour, for the indicated intervals for the different females, was 0.7, 0.0, *0.3, 2.7, 0.7, 0.9, 0.4 per cent., with an average of 0.81 per cent. loss per hour. In the second group with physiologically poor eggs the loss was, 1.0, 3.5, 2.8, 3.3 per cent. per hour with an average of 2.65 per cent. or over three times as rapid.* I cannot account for the only apparent exception, namely female 5, whose rate of loss was 2.7 per cent.

Fig. 1 represents the loss of jelly with age. With physiologically good eggs, the numbers with jelly is a maximum or nearly so, and the rate of loss with age is small, part *a* and *B*. As the eggs become physiologically poor, and in eggs which were in poor physiologic condition at the time of liberation, the numbers with jelly is much less, and the rate of loss with age is more rapid, parts *C*, *D*, etc. It should be noted in passing that parts *B*, *C*, *D* and *E*, are parallel with the corresponding parts of the curve showing change in the volume of eggs with age.

The observations upon *Arbacia* eggs are in entire accord with those of *Hipponoë*. They differ only in the slower rate of loss. One example may illustrate this slower rate. The eggs when freshly removed showed 90 per cent. with their jelly layers. When 41 hours old, 50 per cent. possessed the layer, when 46 hours, 19 per cent., when 70 hours, 1 per cent., and when 72 hours none of the eggs possessed the jelly layer. *In both species of eggs the loss of jelly was a function of age, and physiologic condition at time of the first observation.* If the loss of jelly is known, one can predict (provided proper precautions are taken) the physiologic condition of the eggs and vice versa, from the physiologic condition one may determine the loss of jelly.

Loeb, Harvey and F. R. Lillie in particular, as well as others, have recorded the loss of jelly in old eggs. My observations show not only a loss with age, which is different for different species, but I have determined the rate of loss with age. More than this, I have shown that the rate of loss depends not only upon the species and temperature, but upon the condition of the eggs at liberation, and finally that loss of jelly serves as another symptom of the physiologic condition or vitality of the eggs at any time, and that this vitality diminished at a known rate with age.

Effect of Age upon Membrane Formation.

A third sympton of aging and physiologic deterioration is the change in fertilization membrane. A number of investigators have observed that old eggs do not form fertilization membranes (Hertwig, R. and O., '86, Loeb, '03, '15, Harvey, '10, '14, F. R. Lillie, '14, etc.). Harvey ascertained the exact age in *Arbacia* beyond which, namely 52 hours, no membranes were formed. Just, '15, observed that the jelly layer, which follows fertilization in *Nereis* was retarded with aging.

Before considering in detail the changes in the fertilization membrane, with age, and the causes of such changes, it should be recalled that freshly liberated eggs of freshly collected females, when fertilized under constant and optimum conditions, varied considerably in the rate of membrane formation. Such freshly liberated eggs may be placed into three groups according to their physiologic condition, namely (1) those that form membranes within 2 minutes after fertilization, which are in good physiologic condition. (2) Those that form membranes in 3 to 7 minutes, and which are in moderately poor condition, and (3) those that do not form membranes, which are in poor physiologic condition. It should also be recalled that the rate of membrane formation is further complicated by the differential effect of eggs and sperm. I will mention but two of the experiments, which are typical, to illustrate this differential effect. In experiment 1, Table II., *the eggs of one female were fertilized by the sperm of 5 different males.* All the germ cells were 43 minutes old. Four of the males induced membrane formation in 1½ minutes, the fifth in 2 minutes. Even greater uniformity occurred when the germ cells were 1 hour old, for *the eggs of all 5 samples of the same female formed their membranes at the same time, namely 1 minute.* When the germ cells were 1½ and 2¼ hours old, the eggs again reacted in the same manner for none formed membranes.

In contrast to this uniformity of behavior, when the eggs of one female were fertilized by different males, are those experiments in which the eggs of different females were fertilized by the one male. For example, in experiment 2, the eggs of 3 females were fertilized by the one male, when the germ cells were 20 minutes old. Fertilization membranes first appeared in the three groups of eggs as follows, 3½, 1½ and 1¾ minutes respectively. When the germ cells were 80 minutes old, they appeared in 2⅔, 1⅔, 1⅚ minutes; when 140 minutes old, 1⅔, 1½ and 1 minute; when 280 minutes, ⅔, 1¼ and 1 minute.

In the other experiments similar results were obtained, i. e., uniform or nearly uniform rate of membane formation, when the eggs of one female were fertilized by different sperm, and frequently,

TABLE II.*a.*

SHOWS CHANGE IN RATE OF MEMBRANE FORMATION WITH AGE, OF EGGS OF DIFFERENT FEMALES. *Toxopneustes.*

No.	Date.	Age of Germ Cells in Minutes.	Minutes Required Before first Membranes Appeared.						Average No. Minutes.
			Male 1.	2.	3.	4.	5.	6.	
1	7/3	43	2	1½	1½	1½	1½		1.6
		59	1	1	1	1	1		1.0
		89	N	N	N	N	N		N
		140	N	N	N	N	N		N
			Female 1.	2.	3.	4.	5.		
2	7/14	20	3½	1½	1¾				2.25
		80	2⅔	1⅔	1⅚				2.05
		140	1⅔	1½	1				1.39
		210	1¼	1¼	1¼				1.25
		280	⅔	1¼	1				0.97
		370–600	N	N	N				N
3	7/21	120	1½	1					1.25
		323	1⅓	1					1.15
		450	N	N					N
4	7/16	160	N	N	2½	2½	0	2½	2.5
		300	N	N	N	N	N	1½	0.5
		500–600	N	N	N	N	N	N	N
5	7/7	75	2	3	2½	3½			2.75
		84	1	2	1½	2			1.62
		180	2	2	4½	6			3.62
6	7/12	17	1⅔	1	1				1.22
		42	1½	1	1				1.16
		99	½	¾	¾				0.66
		161	⅓	1	⅔				0.66
		214	⅕	⅔	¾				0.53
		294	2½	⅚	N				1.11
		360	5½	N	N				1.83
		420	N	N	N				N
7	7/19	130	3	3	2	1½	1½	1½	2.08
		240	N	2	2	1⅓	1⅓	1½	1.36
		350	N	2	2	2	2	2	1.83
		470	N	3½	4	N	2½	2½	2.08

N = no membrane in 10 minutes.

wide differences when the eggs of different females were fertilized by the one male. There was a far greater variation among the eggs of different females than among the sperm of different males, or, putting the matter in a slightly different way, the extent of variation in development is conditioned much more by the eggs than by the sperm.

With these facts in mind, we may proceed with the examination of the effect of age upon the membrane formation in physiologically "good," "moderately poor" and "poor" eggs. The data are brought together in Tables II.*a* and II.*b*.

TABLE II.*b*.

SHOWS CHANGE IN RATE OF MEMBRANE FORMATION OF AGEING EGGS AND SPERM.

Arbacia.

No.	Date.	Age of Germ Cells in Hours.		Minutes Required Before Membranes Appeared.						Average No. Minutes.
		♀.	♂.	Female 1.	2.	3.	4.	5.	6.	
1	8/12	4	4	2	1½	3	2	2		2.1
		23	23	3	7	N	N	N		5.0
		28–48	28–48	N	N	N	N	N		N
2	8/14	4	4	2	2	2	2	2	2	2.0
		24	1	N	N	3½	2	N	N	2.7
		31	8	N	N	N	N	N	N	N
		49	1¼	N	N	N	N	N	N	N
		5	5	1½	1½	½	½	½		
3	8/16	1½	1½	1½	1½	1½	1½	1½	1½	1.5
		18½	18½	3½	N	3½	3½	3½	N	3.5
		23	23	N	N	N	N	N	N	N
		24	4	2½	N	2½	N	6	N	3.6
		42	1	N	N	N	N	N	N	N
		48–75.	$\frac{1}{10}$–$\frac{3}{4}$	N	N	N	N	N	N	N
4	8/18	1	23	7	3	3	3			
5	8/19	1	45½	2	N	2½				
6	8/20	5	73	2½	4	4½	5			
		10	95	2½	N	N	N			

N = no membranes in the first 10 minutes.

In the first group, *containing physiologically good eggs, the rate of membrane formation was accelerated with age.* For example in experiment 2, the eggs of the 3 females were fertilized by one male when the germ cells were 20, 80 140, 210, 280, 370, 440 and 550 minutes old. The eggs of female number 1, for instance, formed their membranes at the above ages in 3½, 2⅔, 1⅔, 1¼ and ⅔ minutes, and none after 370 minutes. The eggs of female number 3 formed their membranes in 1¾, 1⅚, 1, 1¼ and 1 minute and none after 370 minutes. The average for all 3 females at the give ages was 2.25, 2.05, 1.39, 1.25, 0.97 minutes, and none between 370 and 600 minutes when the experiment terminated. A corresponding acceleration in the rate of membrane formation, with age, occurred in all the other experiments with physiologically good eggs.

In the second group of physiologically poor eggs, there was also an acceleration in rate of membrane formation with age, but subsequent to this, there was a period of retardation, and ultimate inability to form membranes.

For example in Experiment 5, the eggs of 4 females were fertilized by the same male when the germ cells were 75, 84 and 180 minutes old. The eggs of female no. 1 formed their membranes at these ages in 2, 1 and 2 minutes respectively, in female 3, in 2½, 1½ and 4½ minutes, in female 4 in 3½, 2 and 6 minutes respectively. Female 2 alone did not show any retardation. The average for all 4 females was *2.75, 1.62 and 3.62 minutes respectively.*

In experiment 6, of the three females whose eggs showed an initial acceleration, only one showed a secondary retardation. The observations in this experiment were made at more intermediate ages namely, 17, 42, 99, 161, 214, 294, 360, 420 and 430 minutes respectively. The eggs of female no. 1 formed membranes in 1 ⅔, 1½, ½, ⅓, ⅕ minutes and formed no more membranes after 420 minutes. The average rate for all three females for the corresponding ages, was *1.22, 1.16, 0.66, 0.53, 1.11 and 1.83 minutes* respectively, and none after 420 minutes.

In experiment 7, 5 out of 6 females showed the initial acceleration with age, and the secondary retardation. The average for the 6 females for the following ages 130, 240, 350 and 470 minutes, was *2.08, 1.36, 1.83 and 2.08 minutes respectively.*

The results may be graphically represented as in Fig. 1. The ordinates represent the inverse time or rate of membrane formation. The abcissas represent age of germ cells. With physiologically good eggs, the time and rate of membrane formation decreases with age, parts *A* and *B* of the curve. Beyond certain limits when the eggs are in poor physiologic condition, the time and rate is correspondingly increased, parts *C*, *D*, *E*, etc. In eggs, physiologically poor at the time of liberation, there is either a short period of acceleration, part *B*, followed by a long period of retardation, parts *C*, *D*, *E*, etc. In eggs in still poorer condition, the retardation takes place from the very beginning of the experiment, parts *C*, *D*, *E*, etc. Hence the better the condition of the eggs the longer the membrane-forming period and the longer the period of acceleration. The poorer the condition the shorter the periods.

I have chosen the reciprocal curve, for in this form it becomes quite clear that it is identical with Fig. 1, showing increase in size of eggs, and decreased per cent. of jelly layers, with age. I will discuss this correlation later.

There is one other matter that deserves brief mention. It was observed repeatedly that physiologically good eggs form membranes a considerable distance from the egg surface. With increasing age and with change in rate of membrane formation, the membranes are formed closer and closer to the egg, until they could barely be distinguished from the plasma membrane, and finally did not appear at all. With this reduction in width of the perivitelline space there appeared to be a greater tendency toward scalloping or blistering in the early appearance of the membrane. The Hertwigs in '86 observed a similar narrow perivitelline space and scalloping in ageing eggs.

Arbacia.

Some observations upon *Arbacia* eggs gave similar results, with one exception, namely, there was no initial acceleration of rate in membrane formation so characteristic of *Toxopneustes*. But this lack of acceleration may however have been due to insufficient early observations. The facts are brought together in Table II.*b*.

In experiment 1 (Table II.*b*), the eggs of 2 out of the 5 females that formed membranes at all, after the first observations, showed retardation in rate with age.

In experiment 3, the eggs of 4 out of the 7 females that formed membranes after the first observation, also showed retarded rate of membrane formation.

In experiment 2, one female showed retardation, another showed no change in rate and the other 4 did not form membranes after the first observation.

At this point the question arose whether the inability to form membranes was due to the sperm or to the egg or to both. The matter was tested as follows: *after eggs and sperm of the same age no longer formed membranes*, the eggs were fertilized by fresh sperm, and vice versa, freshly liberated eggs were tested with old sperm. In the first case, *i. e.*, stale eggs fertilized by fresh sperm, fertilization membranes reappeared, and were formed in most instances as rapidly as in previous matings. For example, in experiment 3, membranes ceased to appear when both germ cells were 23 hours old. When 24 hours old, the eggs were fertilized by 4-hour-old sperm, and membranes appeared in 3 out of 7 females. The eggs of 2 females formed membranes in 2½ minutes each, which was more rapid than when the eggs were 18½ hours old. The third formed membranes in 6 minutes. These observations showed that the first inability to form membranes was not due to the absence of membrane-forming substance in the eggs.

When fresh sperm was used with eggs 42, 48, 65 and 70 hours old, no membranes were formed. This showed that at this age the lack of membranes was now due to a change in the eggs which began about 24 hours after liberation in some females, and in 42 hours in other females. The membrane substance was gone.

In the reverse experiments, when increasingly old sperm was used to fertilize fresh eggs, membranes were formed as rapidly and as extended from the surface of the egg as when fresh sperm was used (within certain limitations). For example in experiment 4, 23-hour-old sperm caused membranes to appear in 7, 3, 3, 3 minutes in the 4 females. In experiment 5 the sperm which was 45½ hours old formed membranes in 2 of the 3 females in 2 and 2½ minutes respectively. In experiment 6, when sperm was 73 hours old, membranes appeared in 2½, 4, 4½ and 5 minutes respectively. Even when sperm was 95 hours old (experiment 6) one out of 4 females formed membranes in 2½ minutes.

These and other facts clearly show that *the rate and charac-*

ter of membrane formation, in aging eggs, are functions largely of the egg and determined by their physiologic condition at liberation and by their subsequent age. Physiologically good eggs form clear membranes rapidly with either fresh or old sperm (the maximum age of such sperm was not ascertained). Physiologically poor eggs, form membranes more slowly, closer to the egg surface or do not form membranes at all with either fresh or stale sperm. The change in rate of membrane formation with age, constitutes the third index of the physiologic deterioration of the eggs with age.

Effect of Age Upon Cleavage.

Synchronous Aging of Germ Cells.

Aging or physiologic deterioration may be measured either (1) by a change in size, or (2) a loss of the jelly layer, or (3) a change in rate and character of membrane formation. There is another, more exact and more finely graded index of the changes in the germ cell with age, namely, (4) changes in cleavage.

Hertwig, '96, and Loeb suggested that with age there was a decreasing cleavage. F. R. Lillie, '14, showed in much greater detail that there was a decrease and indicated the extent of the decrease.

In Study I., I showed that under constant and optimum conditions freshly liberated eggs from different freshly collected females frequently differed widely in the total per cent. of cleavage in a given time, even when fertilized by the same male. In *Toxopneustes* the different females vary from 11 to 87 per cent., in *Hipponoë* from 5 to 81 per cent., in *Arbacia* from 0 to 90 per cent. Such differences were ascribed to differences in physiologic condition of the eggs of the different females at the time of liberation.

In preliminary experiments, a given pair of tested sea urchins were used for successive fertilizations. From 100 to 200 of the fertilized eggs were examined at each interval, and the sperm used was freshly prepared from the dry sperm.

In one group of experiments there was an unmistakeable direct reduction in the total cleavage with age. For example, in experiment 1, the eggs of 3 females were fertilized by one male when the germ cells were from ¼ to 6 hours old. The per cent. of the total number of eggs that cleaved at each interval was 98 per cent. when 17 minutes old, 80 per cent. when 42 minutes old, 70 per cent. when 98 minutes old, 33 per cent. when 161 minutes old, 40 per cent. when 214 minutes old, 18 per cent. when 294 minutes old, 0 per cent. when 360 minutes old. The other 2 females showed corresponding decreases. The average for the 3 females was 94, 62, 54, 28, 31, 11 and 0 per cent. respectively. See Table III.

In the other group of experiments, there was an initial increased cleavage in the eggs of some or all of the females, followed by a definite and progressive decrease. For example in experiment 6, the eggs of female 1 and 4 decreased in cleavage with age, as in the first group. But the eggs of females 2 and 3 increased in cleavage for a brief period after the first observation, and then the per cent. decreased. This appeared to me at the time to be an error in observation, but similar increments occurred in other experiments. In experiment 7 the eggs were fertilized at early and frequent intervals, namely, 7, 20, 43, 59, 89 and 140 minutes. All 5 series of mating showed an early *progressive increased cleavage*, followed after 1 hour, by a decrease. The average for all 5 series was 49, 66, 88, 62, 40 and 25 per cent.

It is probable then that in the other experiments the apparent absence of an initial increased cleavage was due to failure to make sufficiently early and sufficiently frequent intervals, or possibly that the increase in cleavage becomes evident only in eggs in poor physiologic condition.

In *Arbacia* only the direct decreased cleavage was observed. Whether an initial increase occurred I cannot say. In the first place insufficient early observations were made, and in the second place, with physiologically good eggs, the maximum or nearly maximum cleavage took place, so that any physiologic change could not be manifested by an increased cleavage.

Only two experiments are given to illustrate the behavior of aging eggs of *Arbacia*. In experiment 8, the eggs of five females fertilized by one male, averaged 80 per cent. when 4 hours old, 33 per cent. when 23 hours old, 0 per cent. when 28½ hours old. (See Table III.)

TABLE III.

SHOWS CHANGE IN TOTAL CLEAVAGE WITH PROGRESSIVE AGE OF GERM CELLS. *Toxopneustes* AND *Arbacia*.

Toxopnuestes.

No.	Date.	Age of Germ Cells in Minutes.	Per Cent. of Eggs that Cleaved.							Time After Fertilization, Minutes.	Average Per Cent. Cleavage.	Per Cent. Loss per Hr.
			Females 1.	2.	3.	4.	5.	6.	7.			
1	7/12	17	98	98	87					120	94	
		42	80	76	31						62	
		99	70	72	20						54	
		161	33	38	14						28	
		214	40	30	23						31	
		294	18	14	2						11	
		360	0	0	0						0	18.0
2	7/19	130	82[1]	96	83	96	98	94		120	91	
		240	93	99	84	89	93	91			91	
		350	92	98	82	64	81	12			71	
		470	77	64	70	4	82	10			51	7.0
3	7/16	160	50	74	81	96	18[1]	14	40	120	53	
		300	34	61	37	92	34	0	50		40	
		500	28	8	20	29	1	6	25		16	6.5
4	7/21	120	51	98						120	74	
		323	11	84							47	8.0
		450	0	0							0	
5	7/14	20	92	47	98					120	79	
		80	99	95	97						97	
		140	98	95	97						96	
		210	96	93	97						95	
		280	92	82	87						87	
		370	62	69	85						72	
		440	82	59	87						76	
		500	43	10	36						29	8.2
		810	0	0	0						0	
6	7/7	75	91	41	67	86				120	71	
		84	75	87	98	80					85	
		180	80	80	17	60					59	7.2
7	7/3	7	80	23	35	68	43			113	49	
		20	95	46	75	73	42			105	66	
		43	80	85	88	95	95			87	88	
		59	62	71	76	19	86			91	62	
		89	38	50	73	31	11			91	40	
		140	34	12	9	11	11			85	25	

Arbacia.

8	8/12	4 hrs.	95	97	18	95	95			60	80	
		23 "	57	49	0	0	60			67	33	2.5
		28½ "	0	0	0	0	0			60	0	
9	8/16	1½ "	100	98	100	88	98	92	100	64	96	
		18½ "	75	20	95	62	88	55	51	60	63	1.8
		23 "	0	0	0	0	0	0	0	60	0	

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[1] Probably an error in recording.

Fig. 1 represents schematically the change in rate of cleavage with age.

The per cent. cleavage is a convenient and finely graded index not only of the physiologic condition of the eggs at the time of liberation, but at subsequent intervals. It became then a simple matter to compute the rate of deterioration with age for a given female, or group of females. The rate of deterioration, *i. e.*, the reduction in cleavage, in per cent. per hour, varied from 6.5 per cent. per hour in experiment 3, to 18.0 per cent. in experiment 1, viz: 6.5, 7.0, 7.2, 8.0, 8.2 and 18 per cent. per hour, for nearly comparable periods. These rates are strikingly greater in *Toxopneustes* (from tropical waters) than in *Arbacia* (of the temperate waters of Massachusetts). Experiments 8 and 9 are illustrative of the rate of deterioration in *Arbacia*, viz: 1.8 per cent. per hour, in experiment 9, 2.5 per cent. per hour, experiment 8. When it is recalled that the difference in temperature of the waters at the two laboratories is about 10° C., and the difference in physiologic deterioration 3 to 10 times as great, it must be evident that the difference in rate is conditioned not only by temperature but by differences in HO concentration, and the egg protoplasm of the different species.

The above data did not make clear whether the decreased cleavage was due to the deterioration of the eggs or of the sperm or both sperm and eggs. The answer to these questions could be obtained in two ways: First to fertilize increasingly old eggs each time with freshly liberated and tested sperm, which would test the rate and degree of degeneration of the eggs. Secondly to fertilize freshly liberated and physiologically good eggs with fresh suspensions of increasingly old sperm, and test the degeneration of the sperm. Both series of experiments were made.

Effect of Age Upon Eggs.

Aging Eggs fertilized by Fresh Sperm. Longevity of Eggs. Toxopneustes.

In the following experiments, eggs and sperm aged synchronously until the eggs either no longer cleaved or only a very small per cent. cleaved. Then samples of the same eggs were fertilized at each subsequent interval by freshly liberated sperm. See Table IV.

In experiment 1, for example, the eggs of 4 females fertilized

Table IV.

Shows Change in Cleavage when Progressively Aging Eggs are Fertilized by Sperm of Varying Ages. *Toxopneustes.*

No.	Date.	Age of Germ Cells in Minutes.		Per Cent. of Cleavage Eggs.						Time in Minutes.	Average Cleavage Per Cent.
		♀.	♂.	Female 1.	2.	3.	4.	5.	6.		
1	7/2	64	64	0	1	0	1			120	½
		209	19	8	85	70	10				43
		250	60	24	80	50	26				45
		309	120	3	44	14	17?				19
		400	3	8	70	2	10				22
2	7/12	420	420	6	2	0				120	3
		436	60	98	92	60					83
3	7/14	500	500	43	10	36				120	29
		600	2	50	36	81					55
		720	720	0	0	0					0
		10	600	33	0	0	10				10
4	7/19a	660	660	0	0	0	0	0	0	120	0
		660	90	95	94	13	32				59
		675	105	10	50	10	12	10	7		16
5	7/19b										
	a)	610	610	0	4					120	
				0	0						
				0	4						1
	b)	640	60	80	8						
				36	50						
				70	25						45
	c)	20	600	47	33	4	23				25
	d)	30	30	18	44	22					28
6	7/16	660	660	5	5					120	5
		7	660					91			91
		670	7	69	54	78					
				78	78						
				40							
		7	7				100				100
7	6/21	660	660	0						120	0
		660	20	18							18
		660	20	13							13
		20	660	0	0						0
		20	20	0	0						0

by the one male, gave a decreasing per cent. of cleavage with age, until the germ cells were 64 minutes old, when only 0, 1, 0 and 1 per cent. of the eggs of each of the females cleaved. The eggs were allowed to age further until they were 209 minutes old, when they were fertilized by freshly liberated sperm, and cleavage rose to 8, 85, 70 and 10 per cent. respectively. This was 42½ per cent. greater than the previous observation. It was clear that the failure to cleave when 64 minutes old was due to the almost complete deterioration of the sperm. It is not clear from this experiment how much the eggs have deteriorated. For as I have shown the results obtained by sperm from different individuals may not be compared without further testing. In this experiment, when the eggs were 225 minutes old, and sperm only 35 minutes old, 32 per cent. cleaved. When the eggs were 250 minutes old and the sperm 60 minutes old, the average cleavage was 45 per cent. Even when the eggs were 400 minutes old, and fertilized by freshly liberated sperm, 22 per cent. cleaved. Hence it is clear that the decline to the zero mark when both germ cells were 64 minutes old, was due not to the death or incompatibility of the eggs, but of the sperm.

Even more striking results were obtained in other experiments. In experiment 2, for example, cleavage decreased progressively until the germ cells were 420 minutes old, and 6, 2 and 0 per cent. of the eggs cleaved. When the eggs 16 minutes later, *i. e.*, 7¼ hours old, were fertilized by sperm only 1 hour old, the cleavage was 98, 92 and 60 per cent. or an increase of 80 per cent. over the last reading. When the eggs of these same females were liberated, and immediately fertilized they averaged 80 per cent. hence it is probable that very little real deterioration of the eggs occurred during the first 7 hours after liberation.

In experiment 3, when the germ cells were both 8 hours old, the average cleavage was 29 per cent., when 12 hours old 0 per cent. When the eggs were 10 hours old and the sperm 2 minutes old, the average cleavage rose to 55 per cent.

In experiment 4, 11-hour-old germ cells gave 10 per cent. cleavage. Eleven-hour-old eggs by moderately fresh sperm gave 59 per cent. cleavage. Fifteen minutes later using the same eggs and sperm, only 16 per cent. cleaved. The marked reduction was due again to the rapid deterioration of the sperm of the second male.

In experiment 5, the eggs of 2 females were tested by 3 different males. When the germ cells were 5 hours old 1 per cent. cleaved. When tested by moderately fresh sperm, cleavage rose to 45 per cent.

In experiment 6, the increased cleavage with fresh sperm was 57 to 73 per cent.

In experiment 7, the small increase with fresh sperm, *i. e.*, only 18 per cent. and 13 per cent. respectively, was due to the poor physiologic condition of the sperm as determined by tests with fresh eggs.

In every experiment, freshly liberated sperm raised the total cleavage from 0 or nearly 0 to 30, 40, 50 and even 80 per cent. of the eggs examined. There can be little doubt but that this means that the apparent death of the eggs (when no cleavage occurred) was really due to the precocious and rapid deterioration of the sperm, with moderately little deterioration of the eggs.

Hence to determine the exact physiologic condition of the eggs at any stage in the ageing cycle, and to determine the real longevity of the eggs, it is necessary to fertilize the eggs with freshly liberated sperm at each testing, as F. R. Lillie, '14, had done for a very different purpose. For example, the eggs of 4 females of experiment 1, Table V., when ⅓ hour old, gave 95 per cent. average cleavage; when 20 hours old, tested by fresh sperm, 67 per cent.; when 23 hours old, 7 per cent.; when 24 hours old, 3 per cent.; when 25 hours old, 1 per cent.; when 46 hours old, ½ per cent.; when 48 hours old, 0 per cent. These figures give a much more exact measure of the rate of deterioration of the eggs than when synchronously aged eggs and sperm

TABLE V.

SHOWS REDUCTION IN CLEAVAGE WHEN AGEING EGGS ARE FERTILIZED BY FRESH SPERM. SHOWS TRUE LONGEVITY OF EGGS. *Toxopneustes.*

No.	Date.	Age of Germ Cells in Hours.		No. of ♂.	Female No.						Average Cleavage, Per Cent.
		♀.	♂.		1.	2.	3.	4.	5.	6.	
1	7/4	⅓	⅓	1	95						95
		20	⅓	2	50	43	95	80			67
		23	1⅔	2	0	4	0	3			2
		24	⅒	4	0	13	0	0			3
		25	1	4	0	1	2	0			1
		46	1	5	0	2	0	0			½
		48	⅒	6	0	0	0	0			0
2	7/5	23	⅒	1	7	0	0				1
		25	1	2	45	0	2				—
		50	⅒	1	0	0	0				0
3	7/7	46	⅓	1	6	0	1	0			1¾
		46	¼	2	0	0	1	0			¼
		47	¾	3	0	2	1	1			1
4	7/8	½	½	1					90	81	85
		23	⅓	1	0	22	43	25			22
		51	⅒	2	0	0	0	0			0

were used. There was very little deterioration or dying until the 20th hour, and then a most rapid rate of destruction until the 24th hour, and a final residual minimum extending until the 48th hour. The eggs of female 1 died at 23 hours female 4 at 24 hours, female 3 at 25 hours, female 2 at 48 hours.

Similar results were obtained in experiments 2, 3 and 4. Forty-eight hours appeared to be the maximum longevity of the eggs of *Toxopneustes* under the given experimental conditions. Table V. gives the rate of deterioration of the eggs, Table III. of the sperm.

Arbacia.

The experiments were repeated with *Arbacia* eggs, and the inquiry was pushed further. The data are brought together in Table VI. The facts may be summarized as follows:

1. Eggs which no longer cleaved when fertilized by sperm of the same age, did cleave when fertilized by freshly liberated sperm. In experiment 1, the increase was from 0 to 39 per cent.; in experiment 2, from 0 to 56 per cent.; in experiment 3, from 0 to 44 per cent., etc. This is exactly as in *Toxopneustes*.

2. With further aging there occurred a second cycle of progressively decreasing cleavage. For example in experiment 5 24-hour eggs fertilized by 1-hour sperm averaged for the 6 females 42 per cent. When the same germ cells were 31 and 8 hours respectively, they averaged only 10 per cent. A similar secondary deterioration occurred in experiment 1. The rapid deterioration was due in both groups to the more rapid destruction of the sperm than of the eggs, as in *Toxopneustes*.

3. The real deterioration of the *eggs* was determined as in *Toxopneustes* by fertilizing aging eggs with freshly liberated sperm at each testing. It was found that the eggs were very long lived. In experiment 7, 20-hour eggs showed little deterioration, 96 per cent. cleaved. Even when 41 hours, there was little deterioration, for 81 per cent. cleaved. When 48 hours 19 per cent., when 65 hours 9 per cent., when 72 hours 1 per cent. The other experiments, viz: 2, 3, 5, 6, 7, 8, 9, 10, 11, etc., gave similar results. They showed the same characteristics as *Toxopneustes* eggs, namely, *a long period of little deterioration, which in Arbacia is about 20 hour under the given experimental*

Table VI.

Shows Reduction in Cleavage when Aging Eggs Are Fertilized by Fresh Sperm. Shows Longevity of Eggs of *Arbacia.*

No.	Date.	Age of Germ Cells in Hours.		Female Number.							Time, Minutes.	Average Cleavage, Per Cent.	Loss Per Cent. per Hr.
		♀.	♂.	1.	2.	3.	4.	5.	6.	7.			
1	8/12a	4	4								60	80	
		28½	28½	0	0	0	0				60	0	
		28½	4½	29	33	28	67				60	39	
		48	½	2	½	0	0				60	1	1.8
		1½	1½								60	96	
2	8/16	23	23	0	0	0	0	0	0	0	60	0	
		24	4	81	79	82	0	65	82	8	60	56	
		42	1	30	0	80	0	0	0	0	90	15	
		42	⅙	77	0	72	0	0	0	0	60	21	
		48	1/10	90	3	79	0	0	0	0	90	24	
		65	¾	48	0	0	0	0	0	0	60	7	
		70	1/12	21	0	0	0	0	0	0	60	3	1.3
		88	1/12	0	0	0	0	0	0	0	60	0	
3	8/17	1½	1½	96	70	97					120	87	
		18½	18½	71	20	93						61	
		23	23	0	0	0						0	
		24	4	28	50	65						44	
		42	1	30	0	17						15	
		42	1/10	66	0	70						45	
		48	1/0	52	3	74						43	
		65	¼	45	4	1						16	
		70	1/12	18	6	5						9	1.1
4	8/17	46	1/12	40	41	67					60	43	
		7	25	0	0	0						0	
5	8/13	4	5	98	97	99	99	98	93		120	97	
		24	1	87	3	57	89	19	0			42	
		31	8	64	0	0	0	0	0			10	3.2
		49	1¼	0	0	0	0	0	0			0	
		49	1¼	0	0	0	0	0	0			0	
6	7/29	20	1/12	73	85						120	79	
		43		34	35							34	
		51		7	5							6	2.3
7	8/1	20–22	1/12	96	96						120	96	
		41		75	87							81	
		48		14	24							19	
		65		3	15							9	
		72		2									1.8
8	8/3	41	1/12	11	22						120	16	
		63		8								8	
		87		0								0	
9	8/9	48	1/12	9	36						120	22	
		68		0								0	
10	8/10	45	1/12	2	7						120	4	
		49		1	7							4	
		51		0	0							0	
		69		0	0							0	
11	8/11	20	1/12	89								89	
		40		7								7	

* Much cleavage, exact count not made.

Table VI.—*Continued.*

No.	Date	Age of Germ Cells in Hours.		Female Number.							Time, Minutes.	Average Cleavage, Per Cent.	Loss Per Cent. per Hr.
		♀4.	♂4.	1.	2.	3.	4.	5.	6.	7.			
12	8/18	6	28	49	99	95	99				90	85	
		1	$45\frac{1}{2}$	0	0	0					60		
				91	47	97					120	81	
		5	$49\frac{1}{2}$	0	17	85					60		
				93	31	77					120	67	
		$\frac{1}{10}$	68	39	90	52	48				60		
				100	100	83	40				120	80	
		5	73	66	74	75	45				60		
				90	69	87	100				120	86	
		10	77	*	*	*							
		$\frac{1}{10}$	95	29	59	72	78				275	59	
13	8/12a	48	48	14							60		
				14							120	14	
	b	18	48	80							60		
				98							120	98	
	c	$\frac{1}{12}$	48	60							60		
				70							120	70	
	d	48	18	11							60		
				13							120	13	
	e	18	18	89							60		
				92							120	92	
	f	$\frac{1}{12}$	18	58							60		
				70							120	70	

conditions. Then there occurred a period of rapid deterioration, i. e., until the 40th hour, and thirdly a long period of minimum cleavage ranging from 50th to the 70th hour. *Arbacia* differs only in the rate of deterioration, which is several times slower than in *Toxopneustes.*

4. The longevity of *Arbacia* eggs was correspondingly longer than *Toxopneustes.* In experiment 1, the eggs of 2 out of 4 females ceased to cleave when 48 hours old. In experiment 2, the eggs of 4 females ceased to cleave at 42 hours; the fifth female at 65 hours, and the 6th female at 88 hours. In experiment 3, cleavage occurred in all 3 females at least till the 70th hour, etc., etc. The longevity ranged from 24 hours to beyond 72 hours, or about twice that of *Toxopneustes* eggs, due largely to the difference in temperature (about 10° C.) of the sea water.

EFFECT OF AGE UPON SPERM.

Freshly Liberated Eggs Fertilized by Aging Sperm. Longevity of Sperm.

In the reciprocal experiments, after the synchronously aging germ cells, no longer cleaved, the aging sperm were tested against freshly liberated eggs.

In experiment 3 (Table IV.), when the *Toxopneustes* germ cells were both 8 hours old, 29 per cent. of the eggs cleaved; when 12 hours old 0 per cent. cleaved. When the sperm were 10 hours old, they were tested by fresh eggs, 10 minutes old, and gave an average cleavage of 10 per cent. This might suggest that the rate of deterioration in synchronously aging sperm cells was conditioned by the rate of deterioration of the sperm.

In experiment 5, 10–hour–old eggs fertilized by 10-hour-old sperm averaged 1 per cent. cleavage. When the sperm was tested by fresh eggs, the cleavage rose to 25 per cent.

Similarly in experiment 6, 11-hour old germ cells averaged but 5 per cent. cleavage; the old sperm by fresh eggs gave 91 per cent. cleavage. On the other hand, in experiment 7, 11-hour-old germ cells gave 0 per cent. cleavage, in both crosses.

The explanation for these apparently contradictory data is found in a triple comparison, namely,

1. Crossing of synchronously aged germ cells.
2. Crossing of aged eggs by fresh sperm.
3. Crossing of fresh eggs by aging sperm.

If this be done it becomes apparent at once that the results are due in largest part to the condition of the egg, subject only to the ability of the sperm no matter how deteriorated to penetrate the cortical layer and initiate development.

In experiment 5, Table IV., 10-hour-old germ cells gave 1 per cent. average cleavage. When the eggs 10½ hours old were fertilized by moderately fresh sperm, 45 per cent. cleaved; the reciprocal cross, viz,, fresh eggs by the old sperm, caused only 25 per cent. to cleave. When these fresh eggs were tested by fresh sperm, 28 per cent. cleaved, *i. e.*, *approximately the same cleavage as when fertilized by 10-hour-old sperm.* Hence low cleavage in experiment *a* was due to the sperm, while in *c* and *d* it was due to the eggs.

In experiment 6, similar results were obtained. Eleven-hour-old germ cells gave 5 per cent. cleavage. Fresh eggs used to test the old sperm gave *91* per cent. cleavage. But when these fresh eggs were tested by fresh sperm, *approximately the same percentage cleaved as when fertilized by stale sperm.*

Experiment 7 is interesting because the fresh eggs used were in physiologically poor condition, and when fertilized by 11-hour-old sperm gave 0 per cent. cleavage. These same eggs tested by freshly liberated sperm gave the same results, namely 0 per cent.

Hence the results obtained by fertilizing fresh eggs by old sperm is determined in largest part by the physiologic condition of the eggs. The sperm plays but a minor rôle. One must be cautious, however, in any given experiment, in interpretng the results. In experiment 3, for example, the 10-hour-old sperm gave only 10 per cent. cleavage with fresh eggs. This low cleavage might have been due to the physiologically poor condition of the sperm or of the eggs. Actual tests showed that the fresh eggs were in poor condition and not the old sperm.

If the sperm deterioration is not reflected, or only to a very slight degree reflected, in a reduction in cleavage how does it happen that in all experiments *when aged eggs were fertilized by aged sperm the cleavage was far less than in either of the other two crosses.*

This result is probably due to a summation of effects, that due to the deteriorated condition of the eggs, and that due to the probable greater difficulty of aged sperm to initiate mitosis and cleavage. In the final analysis both are reducible to the condition of the egg.

Arbacia.

Further light was thrown upon these questions by the experiments with *Arbacia.*

Experiment 12, Table VI., is illustrative of the effect of aging upon the sperm. In this experiment after the germ cells no longer cleaved, the old sperm was tested against fresh eggs of 4 different females, over a long range of intervals. When the sperm were 28 hours old, the eggs of the 4 females averaged 85 per cent. cleavage (90 minutes after fertilization). When the sperm were 45½ hours old 81 per cent. of the fresh eggs of 4 other females cleaved; *when 73 hours old 86 per cent.; when 77 hours old "very many" (exact count not taken); when 95 hours old 59 per cent. of the eggs cleaved.* At this last observation the different females gave 29, 59, 72 and 78 per cent. cleavage. How much longer the sperm could have fertilized freshly liberated eggs was not determined. How much of the reduction (when the sperm were 95 hours old) was due to a physiologic deterioration of the sperm, comparable with that in the eggs, and how much the reduction was due to an excessive mortality, as a result of which there were insufficient numbers of effective sperm, was not determined. Both factors probably play important rôles, though I am inclined to believe that a differential mortality plays a major rôle.

In the above experiment and in others, *the decrease in cleavage with these very old sperm was amazingly small, far less than in eggs of like age.* It should be noted however that the sperm in these experiments were allowed to age in the "dry" or concentrated condition, without the addition of sea water, and in this condition their metabolism is at a minimum, hence the maximum longevity.

In experiment 13, various types of crosses corroborate the above conclusions. Eggs and sperm were fertilized at varying intervals until both germ cells were 48 hours old, at which time only 14 per cent. cleaved. This 48-hour-old-sperm was tested with 18-hour-old eggs and with $\frac{1}{12}$-hour-old eggs. The 18-hour eggs gave 98 per cent. cleavage; the $\frac{1}{12}$-hour eggs 70 per cent. Samples of the same eggs were also fertilized by sperm 18 hours old. The 48-hour eggs gave 13 per cent. cleavage; the 18-hour eggs 92 per cent. and the $\frac{1}{12}$-hour eggs 70 per cent. It will be at once evident that the sperm at both ages gave a *remarkably similar cleavage per cent. with fresh, with moderately old and with old eggs, i. e.*, the sperm have undergone little if any physiologic deterioration between 18 and 48 hours after liberation. The 48-hour eggs gave 14 per cent. cleavage by 48-hour sperm and 13 per cent. with 18-hour-old sperm. The 18-hour-old eggs gave 98 per cent. cleavage with 48-hour-old sperm and 92 per cent. with 18-hour sperm. The $\frac{1}{12}$-hour-old eggs gave 70 per cent. cleavage with 48-hour-old sperm and the same per cent. with 18-hour-old sperm.

The cleavage was determined essentially or exclusively by the condition of the eggs.

The decrease in cleavage in the fresh eggs was due as in *Toxopneustes* experiment to the poorer physiologic condition of the eggs at the time of liberation. *Good fresh sperm cannot cause physiologically poor eggs to cleave to any greater extent than old* *sperm. Cleavage in physiologically good eggs is not decreased by old sperm.*

From these data it will also be observed that the longevity of dry sperm was greater than the eggs in the given experimental conditions. In neither *Toxopneustes* nor in *Arbacia* was the maximum longevity of the sperm determined.

Effect of Age Upon Rate of Cleavage.

I have shown that the total number of eggs that cleaved in a given time after fertilization, decreased as the eggs aged. I propose now to show that there was, as F. R. Lillie, '14, indicated, a corresponding retardation of the rate of early cleavage, with increasing age of the egg; and in a later paper I propose to describe the irregular and abnormal character of cleavage in very aged eggs.

The rate of cleavage was ascertained by the total cleavage at three intervals, namely, (1) 40 minutes after fertilization, when the eggs first divided, (2) 1 hour after fertilization, when most and sometimes all the eggs had divided once, and (3) 2 hours after fertilization when all or very nearly all the eggs had divided at least once.

The data are brought together in Table VII.

In experiment 1, the eggs of 6 females were fertilized by one male, when the germ cells were 130, 240, 350 and 470 minutes old. A record was made at each interval, of the number of eggs that cleaved in 40, 60 and 120 minutes after each fertilization. In female no. 1, 54 per cent. cleaved within 40 minutes, when the germ cells were 130 minutes old; 37 per cent. cleaved in the same interval when 240 minutes old; 3 per cent. when 350 minutes old, 5 per cent. when 470 minutes old. *There was an undoubted decrease in the rate of first cleavage with age. The cleavage at the same ages 60 minutes after fertilization also showed a similar retardation with age of the germ cells*, for 78, 85, 51 and 59 per cent. respectively, cleaved. The total cleavage, namely 2 hours after fertilization, as I have shown before, also decreased with age. The corresponding figures were 82, 93, 92 and 77 per cent.

The average for all 6 females brings out forcibly the retardation and decrease in cleavage. The average for the germ cells 40

TABLE VII.

SHOWS CHANGE IN RATE OF CLEAVAGE WITH AGE OF GERM CELLS. THE FIGURES REPRESENT THE PER CENT. CLEAVAGE IN INDICATED TIME. *Toxopneustes*.

No.	Date.	Age of Germ Cells in Minutes.	Female 1.			2.			3.			4.			5.			6.			Average Per Cent			Ratio of Cleav in 40 Min.: 120 Min.	Ratio of Cleav in 60 Min.: 120 Min.
			40 Min.	60 Min.	120 Min.	40 Min.	60 Min.	120 Min.	40 Min.	60 Min.	120 Min	40 Min.	60 Min.	120 Min.	40 Min.	60 Min.	120 Min	40 Min	60 Min.	120 Min.	40 Min.	60 Min.	120 Min.		
1	7/19	130	54	78	82	70	93	96	73	88	83	82	88	96	86	94	98	91	80	94	76	86	91	83	94
		240	37	85	93	83	99	99	46	56	84	67	76	89	78	78	93	82	67	91	65	76	91	71	83
		350	3	51	92	72	94	98	22	59	82	67	57	64	70	75	89	35	26	12	44	60	71	62	84
		470	5	59	77	17	46	64	16	43	70	0	0	4	64	75	82	1	6	80	27	39	51	53	76
2	7/12	17		98			98			87															
		42	56	77	80	34	78	76	18	30	31										36	62	62	58	100
		99	0	35	70	6	50	72	1	12	20										2	32	54	3	59
		161	4	15	33	6	18	38	1	11	14										3	14	28	10	50
		214	3	24	40	6	28	30	1	11	13										3	21	31	9	67
		294	3	14	18	½	17	14	0	0	2										1	10	11	9	90
		360	0	0	0	0	0	0	0	0	0										0	0	0	0	0
		420	0	0	0	0	0	0	0	0	0										0	0	0	0	0
3	7/14	20	½	93	92	0	32	61	17	98	98										5	74	83	6	89
		80	0	87	99	0	95	95	0	35	70										0	72	88	0	81
		140	2	91	98	2	95	95	2	99	97										1	95	96	1	98
		210	8	83	96	8	63	93	12	97	97										9	81	95	9	85
		280	6	92	92	32	77	82	5	86	87										14	85	87	16	97
		370	6	61	62	4	46	69	3	47	85										4	51	65	6	78
		440	0	25	62	0	20	59	0	51	87										0	32	69	0	46
		500	0	12	43	0	½	10	0	5	36										0	6	29	0	20
4	7/16	160	0	83[1]	50	0	74	74	0	26	81	0	28	96	0	20	18				0	38	53	0	
		300	0	39	34	0	60	61	0	54	37	0	34	92	0	38	34				0	45	49	0	
		500	3		28	0		8	5		20	4		29	1		66				2		26	7	

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[1] Probably an error in recording.

minutes after fertilization was *76, 65, 44 and 27 per cent.* for the 4 different ages of the germ cells. The average cleavage 60 minutes after fertilization, for the same ages, was *86, 76, 60 and 39 per cent.* and the total cleavage, *i. e.*, 2 hours after fertilization, was 91, 91, 71 and 51 per cent. respectively.

These observations suggest an explanation for the decreasing total cleavage with aging germ cells. For the decreased total may conceivably be due to a retardation of the division process, or an inhibition of this process, or both.

Records taken three hours after fertilization showed that the 2-hour observation really was the total cleavage; that very little and usually no additional cleavage occurred. *Hence the decreased total cleavage with aging germ cells was not an apparent decrease due to retardation, but a real decrease by inhibition of cleavage.*

We are concerned then with two symptoms or independent evidences of changes in the eggs caused by their aging. On the one hand, an increasing number of eggs were prevented from developing and, on the other hand, those that do develop, do so with increasing difficulty (retardation).

The relation between retardation and reduction in cleavage may be expressed as follows: When germ cells were 130 minutes old, 83 per cent. of the total number of dividing eggs cleaved within 40 minutes after fertilization; when the germ cells were 240 minutes old a less number, namely 71 per cent. of the cleaving eggs, divided in the same time. When the germ cells were 350 and 470 minutes old, 62 and 53 per cent. of the segmented eggs cleaved in 40 minutes.

In experiment 2, germ cells in poor condition were tested at 8 intervals, between ¼ hour and 7 hours, and the rate of cleavage noted at each interval. The results are essentially in accord with those of experiment 1. The per cent. cleaving in 40 minutes after each fertilization was 36, 2, 3, 3, 1, 0, 0 per cent.; for 60 minutes after fertilization, the record was 62, 32, 14, 21, 10, 0, 0; and the total cleavage 2 hours after fertilization was 62, 54, 28, 31, 11, 0, 0 respectively.

Experiment 4 is also quite in accord with the previous ones.

Experiment 3 differs from the above only in the increasing total cleavage for a brief period, followed by decreasing cleavage with further age. The rate of cleavage follows a similar cycle; *the rate of cleavage and the total cleavage increased with the age of the germ cells until they were 280 minutes old, after which the rate was retarded and the total cleavage declined.*

Arbacia.

With *Arbacia* eggs the rate of cleavage was ascertained by noting the per cent. of the eggs that reached the 4-cell stage in 60 minutes. The data are shown in Table VIII.

TABLE VIII.

SHOWS CHANGE IN RATE OF CLEAVAGE FROM THE PER CENT. OF EGGS THAT REACH THE 4-CELL STAGE IN 60 MINUTES. *Arbacia.*

No.	Date.	Age of Germ Cells in Hours.		Female 1.	2.	3.	4.	5.	6.	7.	Time, Minutes.
		♀.	♂.								
1	8/12	4	4	24	0	0	0	3			60
		23	23	1	0	0	0	1			67
		28	$4\frac{1}{2}$	2	6	5	12	36			67
		24	1	1	0	0	18	1			
		$30\frac{1}{2}$	8	0	0	0	0	0			
2	8/16	$1\frac{1}{2}$	$1\frac{1}{2}$	80	81	90	30	80	65	93	60
		$18\frac{1}{2}$	$18\frac{1}{2}$	3	0	0	0	0	0	1	
		24	4	3	10	1	0	6	1	1	
		42	$\frac{1}{6}$	1	0	0	0	0	0	0	
		48	$\frac{1}{8}$	3	0	2	0	0	0	0	
		65	$\frac{3}{4}$	4	0	0	0	0	0	0	
		70	$\frac{1}{10}$	2	0	0	0	0	0	0	
		88	$\frac{1}{10}$	0	0	0	0	0	0	0	
3	8/14b	$1\frac{1}{2}$	$1\frac{1}{2}$	42	22	67	77	90			60
		5	5	35	3	88	26	40			
4	8/14a	4	5	0	1	0	19	36			60
5	8/18	1	23	0	1	0	0				87
		5	49	0	8	0					60
		$\frac{1}{10}$	68	0	6	0					60
		5	73	5	5	3					60

In *Arbacia* as in *Toxopneustes* the total cleavage decreased and the early division process was increasingly retarded with increasing age of the germ cells. In experiment 3, for example, the eggs of 5 females were tested when their germ cells were $1\frac{1}{2}$ hours old, and 59 per cent. of the eggs reached the 4-cell stage in 1 hour. When the germ cells were 5 hours old only 38 per cent. reached the 4-cell stage in the same time. In experiment 1 and 2, older eggs were used. The results were even more striking and in entire accord with the preceding experiments.

Retardation is another symptom of the deterioration not of the sperm but of the eggs.

Correlation of Changes.

I have shown firstly that the freshly liberated eggs of freshly collected females of the same species varied considerably with respect to size, jelly layer, membrane formation and cleavage; secondly the range of variability for each of these types of changes was ascertained; thirdly, these changes were correlated; fourthly, by *these correlated phenomena one could accurately and conveniently ascertain the exact physiologic condition of the eggs of any female, at any time.*

Freshly liberated eggs from freshly collected females could be readily classified into physiologically "good," "poor" and "bad" eggs, on the basis of the correlated phenomena, as follows:

	Physiologically Good Eggs.	Physiologically Poor Eggs.	Physiologically Bad Eggs.
Size:	Norm for species.	Larger than norm.	Still larger or smaller than norm.
	Small deviation from norm.	Increasing deviation.	Still further deviation.
Jelly layer:	Maximum number with jelly layers 100–90 per cent.	Increasing loss of layer 96–70 per cent.	Further loss 70–40 per cent.
Membrane formation:	Formed within 2 minutes.	Increasingly retarded. 3–7 min.	None.
Cleavage:	Maximum number cleave 100–90 per cent.	Decreasing number 90–60 per cent.	Further decrease. 60–0.

Perhaps one example chosen at random may make the matter more definite. The freshly liberated eggs of *Arbacia* (Experiment 8–12) were practically normal in size, over 90 per cent. contained the jelly layer, fertilization membranes were formed in 1½ to 2 minutes, the cleavage was 95, 97, 96 and 94 per cent. in the eggs of 4 out of 5 females. The eggs of the fifth female were larger than the norm, less than 90 per cent. possessed jelly layers, no membranes were formed upon fertilization and only 18 per cent. cleaved. The eggs of the same females were tested by a second male, and gave the same size and jelly count, as before, and membranes appeared in 2, 2, 2½, 2½ minutes respectively. The cleavage count was 72, 69, 62 and 55 per cent. This retardation in membrane formation and reduction in cleavage was clearly due to the poor physiologic condition of the sperm. The eggs of the fifth female were likewise fertilized by this male and showed the same size and jelly count as before, but no membrane appeared and only 4 per cent. cleaved. By these tests it was definitely determined that the eggs of the first four females were in good physiologic condition, those of the fifth female in poor condition.

By these tests it is possible to state accurately whether any sample of eggs are "good," "poor" or "bad," and to state exactly to what degree of physiologic deterioration such sample of eggs may have reached. The words "good," "poor" or "bad" now have a specific meaning, in terms of definite measurable changes in size, loss of jelly, rate of membrane formation and cleavage, which changes symptomize and measure definite physiologic and morphologic changes in the eggs.

The freshly liberated eggs whose physiologic condition was determined, subsequently changed or aged or overripened, with respect to the same categories, namely, size, jelly layer, membrane and cleavage.

I have shown in this paper exactly the extent to which each of these categories varied with the aging of the germ cells. I wish now to emphasize the fact that the changes are correlated.

These correlated changes, each of which measures the degree of physiologic deterioration with age, may be briefly summarized in the following table:

	Freshly Liberated or Good Eggs.	Moderately Stale or Aged Eggs.	Very Stale or Aged Eggs.
Size:	Little deviation from the norm.	Increasing deviation in a plus direction.	Further deviation in plus or minus direction.
Jelly layer:	Maximum or close to maximum.	Decreasing.	Further decrease.
Fertilization membrane:	Within 2 minutes. Wide membranes.	Increasingly retarded. Increasingly narrow.	None.
Cleavage:	Maximum rate. Maximum number.	Increasing retardation. Decreasing numbers.	Further retardation. Further decrease.

Whatever the physiologic condition of the eggs when liberated, they undergo with age an increase in size, a decrease in the number possessing the jelly layer, a decrease in rate of membrane formation, and a decrease in cleavage, varying in degree with the condition of the eggs at the time of liberation. And one may predict the extent of the other changes from any known one or two. If size or membrane rate are known, one may predict very approximately the other symptoms of the physiologic condition of the eggs, such as jelly and cleavage, etc.

The discussion of these results will be postponed until further data will be presented, concerning other types of changes in very aged eggs. For the present I wish to draw attention to the following considerations.

1. The aging process or processes which are symptomized in the various changes described in this paper begin not with the liberation of the eggs, but upon their maturation, within the body of the mother. This was first suggested by Loeb, and I am in entire accord with his view. Hence it follows that chronologic age (time since liberation) affords but a poor idea of the real physiologic condition of the eggs either at liberation or at any interval thereafter.

2. Aging is a continuous process, beginning within the body and continuing (with somewhat accelerated rate) outside of the body, culminating ultimately in the death of the eggs.

3. Aging was manifested in a number of ways, any one of which may serve as an index of the physiologic condition, and the degree of deterioration. *The ensemble of the various indices forms a clear and unmistakeable measure of their condition and their deterioration.*

4. While no attempt has thus far been made to describe the nature of the chemico-physical processes involved in the aging of the eggs, they may nevertheless be accurately measured. It is now possible to measure very accurately the physiologic condition of the eggs at liberation, and at any interval of time thereafter, to measure accurately the rate of deterioration or senescence under given experimental conditions. It is also possible to measure the real longevity of the eggs or of the sperm.

5. These data, and those described in Part III., afford a basis for an understanding of the nature of the aging process, and of the means of controlling senescence of the germ cells.

The discussion of the results and a more complete bibliography are given in Part III.

SUMMARY.

The variation in size of the eggs, the per cent. with jelly layers, the rate of membrane formation and the total cleavage were ascertained for large numbers of freshly liberated eggs from freshly collected females, examined at different periods of the breeding season.

Three species of sea urchins were studied in this way, namely, *Toxopneustes* and *Hipponoë* of tropical waters, and *Arbacia* of the North Atlantic.

Large variations from the norm were observed and measured in all four categories. These variations were interpreted as indices of the physiologic condition of the eggs of each female at the time of liberation.

With the physiologic condition of freshly liberated eggs of a given female known, experiments were then instituted to ascertain the nature and extent of the changes in the germ cells, as they aged, or became overripe, under given optimum laboratory conditions.

The following is a brief statement of the changes in such aging germ cells, viz., changes in size, jelly layer, membrane formation and cleavage.

Change in Size.

Freshly liberated eggs in good physiologic condition varied but slightly from the norm. For details see text.

As these eggs aged, their volume increased continuously, until they cytolized or fragmented, and became smaller than the norm.

Freshly liberated eggs in poor physiologic condition either enlarged but little with age, or were directly reduced in size by cytolysis or fragmentation.

The nature, extent, and rate of change in size, depends upon the physiologic condition of the eggs when freshly liberated. Whatever the physiologic condition of the eggs may be, their senescence or physiologic deterioration can be very accurately measured by the degree of enlargement or reduction in their size.

Essentially the same results were obtained in the other two species.

Change in Jelly Layer.

Practically all freshly liberated eggs in good physiologic condition possess a jelly layer. Those in poor condition have a correspondingly less per cent.

With age the jelly layer was lost in an increasing number of the eggs.

The rate of loss depended upon the condition of the eggs at the time of liberation (all other conditions remaining constant).

For fresh eggs in good physiologic condition, the rate of loss per hour was 0.81 and for equally fresh eggs in poor condition 2.65 or over 3 times as rapid.

Essentially similar results were obtained in all three species.

The loss of jelly layer was a second symptom and index of the extent and the rate of ageing or senescence of the eggs.

Change in Membrane.

Freshly liberated eggs in good physiologic condition formed fertilization membranes within two minutes. The rate depended partly upon the sperm but primarily upon the physiologic condition of the eggs.

With increasing age the time required to form the fertilization membranes was at first accelerated and later retarded. In very aged eggs no membranes were formed.

Freshly liberated eggs in poor physiologic condition showed direct retardation in the rate of membrane formation.

Aging eggs which no longer formed membranes when fertilized by old sperm could be made to form membranes with fresh sperm.

The rate of membrane formation is practically independent of the sperm. It is essentially determined by the condition of the egg.

As the eggs aged the membrane appeared closer and closer to the surface of the egg; it became thinner and ultimately none was formed.

These observations are essentially the same for all three species.

The rate and character of membrane formation affords a third means of measuring senescence in eggs.

Change in Cleavage.

When freshly liberated eggs of *Hipponoë* and *Toxopneustes* in good physiologic condition were fertilized under the given optimum conditions at successive ages the total cleavage increased for a time and subsequently decreased,[1] and in extreme ageing none of the eggs segmented.

Cleavage then is an additional index of the degree of senescence or physiologic deterioration of the eggs.

There were two series of experiments, one in which the eggs and sperm aged synchronously, and the other in which they aged asynchronously. In both series the rate of decrease in cleavage with age was several times greater in *Toxopneustes* and *Hipponoë* than in *Arbacia*. This difference in rate of senescence as in rate of membrane formation, loss of jelly and change in size is due largely to differences in temperature, as well as to differences in HO concentration of the sea water, and to protoplasmic differences of the eggs.

When both germ cells aged synchronously the apparent longevity was about 11 hours for *Toxopneustes* and about 28 hours in *Arbacia*.

In asynchronous matings a more definite idea was obtained of the changes in the egg alone and in the sperm alone. When freshly liberated sperm were used to fertilize the eggs (of a female) at varying ages, with the precautions indicated in the text, the eggs showed progressively decreasing per cent. of cleavage (in *Arbacia*), but the rate of decrease was very much slower than when both germ cells aged synchronously. The decrease may be divided into three periods, the first a period of small decrease (about the first 20 hours in *Arbacia*) the second, a period of rapid and large decrease (between the 20th and 40th hour), and the third, a period of small decrease (between the 40th and 80th hour in *Arbacia*).

Eggs in poor physiologic condition at the time of liberation deteriorated at a correspondingly greater rate than physiologically good eggs.

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[1] Freshly liberated eggs, of freshly collected *Arbacia*, in good physiologic condition, gave a maximum or nearly maximum cleavage and with ageing there was a direct decreasing total.

The real longevity of the eggs depended upon the physiologic condition at time of liberation, the better, the longer lived, and vice versa.

The rate of cleavage was accelerated for a time and then retarded with age, more so in physiologically poor eggs, less in good eggs.

When ageing sperm fertilized freshly liberated eggs the decrease in cleavage was surprisingly small. Very little decrease occurred when sperm were 73 hours, only a little more when sperm were 95 hours old.

The explanation for the greater longevity of the sperm is found in the fact that the sperm aged in the "dry" condition, in which they are inactive and hence minimum metabolism.

The greater decrease in cleavage in synchronously ageing germ cells is due to a summation of the injurious effects upon the sperm and upon the eggs.

In very late stages in ageing of sperm, the reduction may be due not so much to physiologic deterioration as to insufficient numbers of sperm. There is ground for belief that no matter how old or deteriorated the sperm if they are active (alive) they can penetrate the egg. It is not so clear whether such aged sperm cause parthenogenesis or sexual development.

The change in size, jelly, membrane and cleavage with aging of germ cells, are accurate, convenient and corroborative indices of chemico-physical and morphologic changes in the egg as they age, and afford convenient measures of the loss in vitality, or physical deterioration. And one change may serve for this purpose. Their ensemble is convincing.

In the next study will be considered the changes in much older germ cells leading towards their cytolysis and death.

[illegible]

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